

Students' Use of Diagrams for the Visualisation of Biochemical Processes

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This dissertation is dedicated to my parents Kevin and Audrey Hull,
as a token of appreciation for their belief in me and affording me the opportunities
to follow my own path

Preface

The research described in this dissertation was carried out in the Science Education Research Group (SERG), Discipline of Biochemistry, School of Molecular and Cellular Biosciences, University of Natal, (Pietermaritzburg), from August 2000 to September 2003 under the supervision of Dr Trevor R. Anderson and the co-supervision of Prof Diane J. Grayson (University of South Africa, Pretoria).

These studies represent original work by the author and have not otherwise been submitted in any other form for any degree or diploma to any other university. Where use has been made of the work of others, it has been duly acknowledged in the text.



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Abstract

Research into the usefulness of scientific diagrams as teaching and learning tools has revealed their great effectiveness in reinforcing and replacing text; summarizing, clarifying, grouping and comparing information; illustrating abstract concepts and spatial relations between concepts; and aiding understanding and integration of knowledge. However, these advantages are not always realised as diagram effectiveness depends on the student's cognitive ability, visual literacy and prior knowledge.

In biochemistry, flow diagrams are used as tools for the visualisation of biochemical processes, the abstract nature of which presents problems to students, probably because the depicted content is beyond their perceptual experience. In this study, we define visualisation as the entire process from the perception of an external representation (e.g. diagram), its internal processing, and the expression of a mental model of the represented content. Therefore, visualisation incorporates reasoning processes and interactions with a student's conceptual knowledge, in their construction of a mental model. Students' visualisation difficulties, in terms of conceptual and reasoning difficulties, have been well researched in areas such as physics and chemistry, but neglected in biochemistry, especially with respect to the use of diagrams as visualisation tools. Thus the aim of this study was to investigate students' use of diagrams for the visualisation of biochemical processes, and to identify the nature, and potential sources of students' conceptual, reasoning and diagram-related difficulties revealed during the visualisation process.

The study groups ranged from 27 to 95 biochemistry students from the University of Natal and 2 to 13 local and international experts. Propositional knowledge was obtained from textbooks and from a questionnaire to experts. Data on student visualisation of biochemical processes was obtained from their responses to written and interview probes as well as student-generated diagrams. All data was subjected to inductive analysis according to McMillan and Schumacher (1993) and any difficulties that emerged were classified at levels 1- 3 on the framework of Grayson *et al.* (2001). The possible sources of difficulties were considered in terms of a model by Schönborn *et al.* (2003 & 2002).

The results revealed the following major findings. The meaning of linear, cyclic and cascade biochemical processes was partially resolved by means of an extensive list of generic and distinguishing functional features obtained from experts. Attempts to clarify propositional knowledge of the complement system revealed a deficiency in our understanding of the functional relationship between the complement pathways and highlighted the need for further experimental laboratory work. Several students literally interpreted diagrams of the functional characteristics of biochemical processes (e.g. cyclic) as the spatial arrangement of the intermediates within cells (e.g. occur in "circles"), although in some cases, their verbal responses revealed that they did not hold this difficulty suggesting that they might hold more than one internal model of the process. Some students also showed difficulty using textbook diagrams to visualise the chemistry of glycolytic and complement reactions. In this regard, besides students' conceptual knowledge and reasoning ability, a major source of these difficulties included misleading symbolism and visiospatial characteristics in the diagrams, suggesting the need for improvement of diagram design through the use of clearer symbolism, the standardisation of conventions, and improvement of visiospatial properties of diagrams. The results constituted further empirical evidence for the model of Schönborn *et al.* (2003 & 2002) and led to the proposal of a model of visualisation aimed at clarifying the highly complex and cognitive processes involved in individuals' visualisation of biochemical processes in living systems.

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List of Abbreviations

ATP	adenosine triphosphate
ADP	adenosine diphosphate
C	designates a complement protein
DHAP	dihydroxyacetone phosphate
EC	enzyme cleavage
G3P	glyceraldehyde 3-phosphate
I	interviewer
IR	isomer reaction
LI	literal interpretation
pers. comm.	personal communication
S	student
SR	split reaction

CHAPTER 1

Introduction and Aims

The “successful visual representation of large bodies of information is the key to success in the information and communication age” in which we now live (Fisher *et al.*, 2000). Thus, highly illustrated forms of information are increasingly being used in instruction in many disciplines (Lowe, 2003), including in biochemistry. It follows, that research into the effectiveness of diagrams and other visualisation tools is one of the most rapidly expanding areas of science education research.

Research into the general effectiveness of diagrams in the instruction and learning of science has revealed a wide range of positive effects of diagrams including their role in reinforcing and replacing text, summarizing, clarifying, grouping and comparing information (e.g. Schnotz, 1993, 1993b & Holliday, 1990). Diagrams have also proved useful in illustrating abstract concepts by providing an analogy (Bean *et al.*, 1990 & Hurt, 1987). In addition, the ability of diagrams to show spatial relations between the concepts and the objects that they illustrate, decreases the amount of search required to find information and the number of words required to explain these spatial relationships (Glenberg & Langston, 1992, Larkin & Simon, 1987 & Winn & Holliday, 1981). Furthermore, the cognitive effects of diagrams in instruction include aiding integration of new knowledge into existing knowledge structures, supporting cognitive processing, freeing and expanding the functional capacity of the working memory, retention functions and facilitating and encouraging problem solving (e.g. Schnotz, 1993, 1993b, Glenberg & Kruley, 1992 Holliday, 1990, Levie & Lentz, 1982 & Dwyer, 1969). These reported advantages of diagrams are, however, not always realised when students use abstract diagrams, for example, flow diagrams representing biochemical processes. This is believed to be because the depicted content in abstract diagrams is beyond our perceptual experience and are, therefore, often more difficult to process (Lowe, 1994 & 1993 & Alesandrini & Rigney, 1981). In addition, factors such as cognitive overload need to be taken into account when students process diagrams and dynamic visuals (e.g. Lewalter, 2003, Lowe, 2003, Mayer, 2003 & Stern *et al.*, 2003). Thus, the effectiveness of diagrams in instruction and learning depends on various factors such as the age and ability of the

students, what is being assessed, e.g. recall or understanding (e.g. Winn & Sutherland, 1989), the amount of prior knowledge an individual brings to the diagram, as well as their degree of visual literacy (e.g. Pintó & Ametller, 2002 & Lowe, 1999).

In addition to the above, one of the primary uses of diagrams in instruction is their role as visualisation tools. In this regard, research into their effectiveness as visualisation tools has extended from studies on static diagrams, to the effectiveness of more dynamic visuals on student learning. For example, animations are believed to aid students in constructing adequate mental representations, as they promote students' visualisation of spatial aspects of dynamic and complex biological processes (Lewalter, 2003 & Nerdel *et al.*, 2002). However, Lewalter (2003) suggests that static visuals are superior to animations as the learner can control the speed of their learning, and construct their own internal visual models of the dynamic process. In addition, Nerdel *et al.* (2002) found that comprehension on biological processes was better understood using static diagrams rather than animations. Thus through the combination of static diagrams and animations, we get interactive graphics, that allow the learner to work at their own pace (Richardson and Richardson, 2002) and at the same time, also provide the power of dynamic animations as visualisation tools.

In our context, biochemical processes are complex and dynamic (Nerdel *et al.*, 2002) and because they are abstract, our understanding of them is not based on direct experience, which contributes to the difficulties students have with understanding them (Kindfield, 1993/ 1994 & Flores *et al.*, 2003). In terms of visualising these processes, Kindfield (1993/ 1994) has suggested that diagrams and diagram skills are vital components of what is learnt about biological or biochemical processes. In this regard, Britton and Wandersee (1997) state that understanding of biological process is promoted through the use of diagrams, as students have stated that they have better retention and form better mental models through the use of illustrations. In terms of the diagrams used to depict biochemical processes, students still have no realistic means with which to visualise the process (Lowe, 1986). This is because the diagram elements often do not represent their real life counter parts and the function of this type of diagram is, therefore, to depict the relationships between the elements (Lowe, 1986). Literature reports on student difficulties with visualisation, especially in the context of biochemistry, are limited. Some examples are, students' difficulties visualising IgG (immunoglobulin G) molecules (Schönborn *et al.*,

2002), difficulties in visualising processes depicted by symbolic conventions such as arrows (Amettler & Pintó, 2002, du Plessis *et al.*, 2002 & Hull *et al.*, 2002), difficulties in spatial visualisation of biomolecules (Makura *et al.*, 2000) and biological sections (Sanders, 1995), and difficulties with the visualisation of macro-micro symbolism in chemistry (Johnstone, 1991).

Perhaps the reason why there is not much literature in terms of students' visualisation difficulties, especially in the context of biochemistry, is because the term "visualisation" is not well defined. The everyday meaning is to "see a mental image" (Sanders, 2003, pers. comm.), although the literature also contains many different meanings of the term "visualisation". For example, Schnotz (2003, pers. comm.) views "visualisation" to be the process whereby a picture or image is formed from a pre-existing mental model, i.e. from information that has already been understood and is stored in memory. In contrast, for the purposes of this dissertation, we adopt the definition of Anderson (2003, pers. comm.) and McCormick *et al.* (1987), who view visualisation more broadly as involving the perception, interpretation, use and communication of visual information. Thus, this encompasses the entire process from reading/ perceiving a diagram, internally processing it by "filtering" and integrating it with conceptual knowledge thereby constructing a mental model, and subsequently externally expressing an interpretation of it (Anderson, 2003, pers. comm. & McCormick *et al.*, 1987). In addition, Franco *et al.* (1999) state that mental models involve visualisation as it supports scientific thinking and the use and development of mental models.

The manner in which new, selected information is processed and integrated into an individual's cognitive structure or mental model can be explained by Mayer's dual coding theory (e.g. Mayer, 2003). Mayer and Anderson (1992) have described the dual coding theory to be the manner in which information is processed by either of the two distinct information processing systems possessed by humans, i.e. one represents information visually and the other represents information verbally. For example, visual information in the form of a diagram will be processed in the visual channel, and verbal information in the verbal channel. Referential connections exist, between the visual and verbal representation systems in the working memory, and these connections allow the integration of the two representation systems, to allow the construction of a runnable mental model (e.g. Mayer, 1993 & 1992). In this regard, Schnotz (1993b) remarked that the visual and verbal codes

complement each other as they are qualitatively different and enable human cognition to be highly efficient. Mayer (2003 & 1997) has subsequently elaborated on his dual coding theory and Schnotz and Bannert (2003) have further modified this theory in terms of multimedia learning (Chapter 2, Section 2.3.3).

When constructing a mental model from a diagram, the user needs to visually process the diagram at a number of levels. The first stage is perceptual processing whereby the diagram is classified on a visuospatial level i.e. the elements making up the diagram are processed (see Chapter 2, Section 2.2.1), the second stage or semantic classification involves relating the diagram elements into what they represent functionally and thereby creating a propositional representation as well as a mental model (Humphries & Bruce, 1989 & Schnotz & Bannert, 2003). Therefore in order for a diagram to be effective, it should lead to the construction of a mental model based on semantic classification of the diagram. This would occur via the mapping of diagram elements onto mental entities and mapping spatial relationships onto semantic relations (Schnotz & Bannert, 2003), which would be challenging for novices who lack prior knowledge and may therefore build mental models based purely on the visiospatial characteristics of the diagram (Lowe, 1993).

The successful use of diagrams as visualisation tools is largely influenced by the student's conceptual knowledge and their reasoning ability (Schönborn *et al.*, 2003 & 2002). In this regard, research by various authors has highlighted that diagram interpretation is not simple, especially when conceptually challenging information is portrayed, and when students have a limited conceptual knowledge (e.g. Stylianidou *et al.*, 2002 & Lowe, 1999). In this respect, research into students' use of diagrams has shown that a lack of prior or conceptual knowledge (e.g. Lowe, 1999 & Winn, 1993) and the use of everyday dynamics and everyday knowledge, sometimes incorrect, may lead to the formation of an inappropriate mental model and limited inference making from the diagram (e.g. Cheng *et al.*, 2001, Lowe, 1996 & 1999 & Pintó & Ametller, 2002). For example, Lowe (1996 & 1994) found that experts, who have a more extensive conceptual knowledge than novices, were able to make inferences by using a diagram in conjunction with their prior knowledge. In contrast, he found that novices tended to be restricted to the visiospatial elements of the diagram (Lowe, 1996 & 1994). The numerous strategies that the experts would have employed in the reasoning task are beyond their conscious control and,

therefore, they may have difficulty remembering the strategies once the task is completed (Lewalter, 2003). Clearly, the importance of conceptual knowledge and reasoning ability is related to the success an individual has with interpreting a diagram. Thus, Schönborn *et al.* (2003 & 2002) investigated the effects of students' conceptual knowledge, their reasoning ability and the mode of representation or diagram itself, on students' interpretation of diagrams (Chapter 3, Section 3.3.8). These authors stated that it is seldom possible to attribute a student's difficulty with interpreting a diagram solely to one of these factors and that an interaction between one or more of the factors is often found (Schönborn *et al.*, 2003 & 2002).

The role of conceptual knowledge in student learning can conveniently be explained by the constructivist theory (e.g. von Glasersfeld, 1992). Constructivists view learning as an active process in which new ideas or concepts are constructed based upon a learner's prior knowledge (Kearsley, 1993-2003). This active or meaningful learning involves the relevant selection, transformation, interaction and integration of new knowledge, with the existing or prior knowledge a student already possesses, into a coherent cognitive structure for permanent storage (Mayer, 1989, Driver & Bell, 1986 & Pines & West, 1986). A cognitive structure (i.e. schema, mental model) has been referred to as the manner in which information is organized in the human memory and it allows an individual to make inferences or "go beyond the information given" (Kearsley, 1993-2003 & Jonassen & Hawk, 1984). Thus, a cognitive structure serves as the framework on which to anchor new knowledge (Pearsall *et al.*, 1997).

However, students do not always construct scientifically acceptable conceptions when they are presented with new information. In this regard, alternative or misconceptions disagree with the currently accepted conceptions of experts in the field, although, in some cases, they may stem from ideas of early leaders in the field (Fisher, 1985). These alternative conceptions may be constructed in a number of ways when there is a conflict between new ideas or concepts and what was previously believed (Pines & West, 1986). In cases where there is a reluctance to abandon previous conceptions by students, alternative conceptions may be constructed and persist (Pines & West, 1986). These alternative conceptions may be constructed in a number of situations, from experiences that are common between many individuals, through school and other teaching, or through incorrect language processing (Fisher, 1985). Assumptions have been made that alternative conceptions are more likely

to arise when an individual is first confronted with scientific data, as this is the beginning of the cognitive restructuring process, and incorrect preconceptions may hamper the integration of new information into an individual's mental model (Spada, 1994). In general, alternative conceptions are held by a great number of individuals and many are difficult to correct through traditional teaching strategies (Fisher, 1985).

Since alternative conceptions are reported to seriously interfere with science learning (Treagust, *et al.*, 1996), their identification and remediation is a very important goal of science education research. In this respect, there is an expansive amount of diverse literature available on different student conceptual and reasoning difficulties in various disciplines, including physics (e.g. Harrison *et al.*, 1999), chemistry (e.g. Garnett *et al.*, 1995), mathematics (e.g. Wong *et al.*, 2002) and biology (e.g. Fisher, 1985). However, substantially less research has been done in the context of biochemistry (e.g. Anderson *et al.*, 1999). This literature is readily made available to researchers and teachers through databases such as ERIC (<http://ericir.syr.edu/Eric/>) that houses many journal articles and links to other websites, and CARD (conceptual and reasoning database, www.card.unp.ac.za), which also provides conceptual questions, categories of difficulties and suggested remediation strategies. Other sources of science education literature are also available, for example, in the form of a comprehensive bibliography by Pfundt and Duit (1994).

Returning to the context of biochemistry, limited education research has been done, especially in terms of diagram research. For example, Nunez de Castro and Alonso (1997) looked at students' interpretations of diagrams depicting enzyme-catalysed reactions and Menger *et al.* (1998) used diagrams of micelles in their study of student understanding. More generally, Anderson and co-workers (e.g. Anderson *et al.*, 2002 & Anderson *et al.*, 2000) have identified various student conceptual and reasoning difficulties with the understanding of the glycolytic pathway, which might be applicable to other metabolic pathways. In the field of immunology, Simonneaux (2000) investigated students' conceptions of the immune system and Schönborn *et al.* (2002) have investigated the sources of difficulty some students have with the interpretation of a range of diagrams representing an IgG molecule. Other authors have suggested various experiments to illustrate biochemical concepts in a laboratory (e.g. immunological concepts (Shulman *et al.*, 1999) & protein structure (Noriega & Vazquez, 2000)), or suggested various teaching

approaches to be used (e.g. to aid student learning about metabolism (Punekar, 2000)). There is, therefore, limited biochemistry education literature available, especially in terms of diagrams, which are the primary tools used to teach biochemical processes.

Why do we want to investigate students' use of diagrams for visualising biochemical processes? This introduction has highlighted the deficiencies that exist due to the limited science education research that has been done on students' conceptual and reasoning difficulties in the field of biochemistry as a discipline. Since biochemical process information is primarily communicated through the use of abstract diagrams, the effectiveness of these diagrams in communicating information to students is an important area of investigation. However, due to the mixed reports on the effectiveness of diagrams as teaching and learning tools in science in the literature, we therefore aim to provide experimental evidence either in support, or against, the use of diagrams in the instruction and learning of biochemical processes. At the outset, it would seem that biochemistry is a minefield of unidentified student difficulties because as Anderson *et al.* (2002, pers. comm.) have pointed out, metabolism requires students to transfer and integrate their content knowledge and reasoning from several areas of biochemistry. Through exploring this avenue of biochemical education research, we hoped to gain insight into how students visualise biochemical processes as a result of exposure to diagrams. The implication of this type of study, will be that we will be able to compile a list of guidelines for diagram designers so that they may design better/ more effective diagrams, as well as be able to suggest possible remediation and intervention strategies.

Thus, the aim of the studies reported in this dissertation was to investigate students' use of diagrams for the visualisation of biochemical processes, and to identify the nature, and potential sources of students' conceptual, reasoning and diagram-related difficulties revealed during the visualisation process. From these broad aims, we formulated the following research questions:

1. What is experts' understanding of the distinguishing functional features of linear, cyclic and cascade biochemical processes and what recommendation can be made for the description of these processes in textbooks and the literature?
2. How do students' visualise the spatial arrangement of biochemical processes occurring within cells?

3. How do students' visualise the chemistry of the individual reactions in a biochemical process, represented by a textbook diagram?
4. What influence do textbook representations of biochemical processes have on students' ability to visualise these processes?
5. Do students show any visualisation difficulties and, if so, what is the nature of the difficulties?
6. What are the possible sources of these visualisation difficulties?
7. What are the implications of this research for improving student visualisation of biochemical processes?

The above research questions are addressed in various results chapters in this dissertation. After reviewing the literature (Chapter 2), Chapter 3 presents the research process and the rationale for the methods used in the various studies, reported in Chapters 4- 7. In Chapter 4, we present expert understanding of the distinguishing functional features of linear, cyclic and cascade biochemical processes, followed by an investigation of students' visualisation of various biochemical processes in Chapters 5- 7. Chapter 5 investigates students' visualisation of the spatial arrangement of biochemical processes occurring within cells, while Chapters 6 and 7 focus on their visualisation of the chemistry of individual reactions, using 2 textbook diagrams representing the glycolytic and complement pathways, respectively. Finally, Chapter 8, consists of a synthesis and general discussion of the findings of the studies reported on in Chapters 4- 7, and suggests a possible model for the visualisation process, as well as avenues that are open for future research opportunities.

CHAPTER 2

Literature Review

Terms such as diagrams, illustrations, diagrammatic representations, visual displays/ aids, graphic displays/ aids, pictures, images, sketches, drawings, figures... are often used interchangeably in the literature on diagram research. In most cases, the definitions and contexts within which they are used, are seldom clear or constant. The common theme of the studies reported in this dissertation was diagrams as visualisation tools, with the primary focus being on the identification of students' understanding of, and visualisation difficulties with, biochemical processes (Chapters 5- 7). Therefore, in a broad overview this literature review focuses on firstly, what are diagrams? (Section 2.1); secondly, the reported effectiveness of diagrams in the instruction and learning of science (Section 2.2); and thirdly, the meaning and process of visualisation and mental model construction through the use of diagrams as visualisation tools. Included is a consideration of the roles of conceptual knowledge and reasoning with diagrams, for visualisation (Section 2.3). By considering these themes, I hope to provide a focused and comprehensive account of the current status of diagram research and the opinions of various authors. In doing so, I aim to highlight deficiencies and/ or discrepancies in the diagram research field, as a motivation for the current studies reported on in this dissertation.

2.1 What is a Diagram?

Since the objective of this dissertation was to investigate students' understanding and visualisation of biochemical processes and, therefore, their understanding of the diagrams that depict these processes (since they are the primary tools for communicating this information to the students), it is important to try to define the term "diagram". Blackwell (2001) states that diagrams are neither linguistic, such as speech and written text, nor pictorial representations; while Lowe (2001, pers. comm.) states that it is not easy to produce a neat definition of a diagram in the context of science, because there is such a wide range of graphics that are described as "diagrams". None-the-less, in this section we

mention some of the criteria various authors use as an attempt to bring clarity to the broadly used term “diagram”.

It seems logical to start with a dictionary definition of “diagram”, defined as, a “sketch showing the features of an object needed for exposition; graphical or symbolic representation, by lines, of process, force, etc.; (Geom.) figure made of lines used in proving theorem etc...” (Concise Oxford Dictionary, 1982, pg 264). The Concise Oxford Dictionary, therefore, classifies diagrams in terms of what is being depicted and that their content may be depicted in a number of external forms. In terms of defining diagrams based on their general composition and depiction, there is both correlation and disagreement between certain authors in different disciplines. For example, diagrams have been agreed to be schematic pictures of objects representing concepts; where conventions are used to interpret the symbols (Kosslyn’s, 1989). In addition, the depicted spatial relationships between the objects can be expressed by statements, making the diagram abstract and propositional (Do & Gross, 2001). Despite some correlation between the definitions of certain authors, there are however some discrepancies when defining a diagram on the criteria of what they depict. For example, while some author’s class maps as diagrams (e.g. Lowe & colleagues), other authors separate these into two representational systems, and say that diagrams do not represent parts of a territory as do maps (Kosslyn, 1989). Consequently, “maps show spatial relationships, [whereas] diagrams show conceptual relationships” (Levie & Lentz, 1982).

It is possible, however, to depict spatial relationships in diagrams, and some authors classify diagrams based on their structural or visiospatial characteristics. For example, many authors consider the two-dimensionality of a diagram in their definitions (e.g. Hardin, 1993 & Fry, 1981), and use this feature to separate diagrams from text, where all the elements appear in a single sequence, i.e. a sentential representation (Larkin & Simon, 1978). Other authors make use of the visiospatial characteristics of the elements within the diagram to define a diagram, since these elements represent the physical objects, processes and interrelationships in the real world (Lowe, 1993b & Lohse *et al.*, 1991). These elements comprising the diagram are the smallest units within the diagram and are either contextual (they aid the user in interpreting the functional elements), or functional (they depict the message of the diagram) (Lowe, 2001, pers. comm.). The elements can be grouped or distinguished from each other by perception (Cheng *et al.*, 2001). The

dimensionality, elements of the diagram, and their spatial arrangement, have all been taken into account by Stenning and Lemon (2001), who describe diagrams as plane structures where the depicted spatial relationships can be interpreted as relationships in the real situation.

The above-mentioned visiospatial characteristics of diagrams are due to the *transformations* that are used to depict the original object, concept or realistic situation. Often, this causes diagrams to fall on a continuum from realistic to highly abstract representations, which means that more abstract diagrams have undergone more *transformations*. Various transformations are required in order to represent some content diagrammatically, for example the representation of various non-spatial dimensions, such as a sequence of events or processes, to be represented spatially (Lowe, 1993b). This approach is applicable to the diagrams used in the studies reported in this dissertation (Chapters 5- 7), where flow diagrams are used to depict an abstract biochemical process. It follows therefore, that diagrams have been described as line drawings with features or transformations such as abstraction, simplification and conventions (Lowe, 2001, pers. comm.), to allow the expression of spatial data describing the structure of physical objects, or interrelationships and processes associated with the physical object (Lohse *et al.*, 1991).

Moving away from the characteristics of the diagram itself, contradictory views on what characterises a diagram are also found when the effects of the diagram on the diagram user are taken into account. Schnotz (1993b), in light of the dual coding theory, views graphics as visual codes where elements and relationships on the graphic can be mapped onto a mental model. Alternatively, Larkin and Simon (1987) view a diagram as an external model rather than an internal mental model. They believe a representation (of any nature) is made up of data structures that are stored externally (e.g. on paper) (Larkin & Simon, 1987). Thus, “programs” (stored in memory) act on the data structures to allow the individual to make inferences from the external representation (Larkin & Simon, 1987). The further investigation into what constitutes a mental model (defined as representations of how individuals understand and interpret the world (Winn, 1991)), and the imagery debate, (which considers the nature of images as mental representations), are discussed in greater detail in Sections 2.3.3 and 2.3.3.1 respectively.

It is therefore evident from the range of studies reported, that there is still no clear definition of a diagram. However, in trying to move towards clarifying what defines a diagram, many authors have proposed taxonomies for diagrams and their information content (e.g. Shimojima, 2001, Mandler & Parker, 1976, Mandler & Johnson, 1976). In light of the contradictory definitions and criteria (some authors use a range of criteria) for defining what a diagram is, we believe that in diagram research it is important to define a diagram in the context of the study as there is to date no, and there may never be, succinct or operational definition of a diagram illustrating the continuum of representations from text to highly abstract diagrammatic displays.

The lack of clarity on the meaning of a diagram also pertains to the different types of diagrams. Cheng *et al.* (2001) acknowledge this lack of clarity and state that there are many types of diagrams (e.g. flow diagrams, geometric diagrams and maps) that fall on a continuum that includes other representations such as mathematics equations. Reasons for this continuum of representations, might be that hybrids of diagrams exist whereby more than one type of diagram is present within a particular representation (Kosslyn, 1989); or that a particular representation may contain a mixture of characteristics specific to various types of representations, e.g. some diagrams contain propositions, mathematics equations, and spatial characteristics (Cheng *et al.*, 2001). It is, therefore, futile to place diagrams into set types or categories, and the point made previously in this review that authors need to define their definitions of a diagram in the context of their studies, is reiterated.

The diagrams used in this study for investigating student understanding and visualisation of biochemical processes (Chapters 5- 7), are of the flow diagram or flow chart type of diagrammatic display. We use the words “type of diagrammatic display” as there is no clear-cut definition of a diagram, and therefore, although we are considering a type of diagram when looking at flow diagrams, there may still be features that are, or are not, characteristic of *all* flow charts or diagrams.

The content depicted by flow diagrams (e.g. biochemical processes) is often beyond the viewer’s immediate or perceptual experience and, although conceptually or logically related, flow diagrams do not resemble the objects or process that they are depicting (Lowe, 1997 & Alesandrini, 1984). This is due to the extensive external manipulations that have been done and, for these reasons; Lowe (1997) and Alesandrini (1984) place flow

diagrams into an arbitrary or abstract diagram category. There is also a difference in the structure of text and flow diagrams. In text, the linkages between concepts are words or sentences, and in flow diagrams these linkages are condensed in the form of lines or arrows that, through their spatial integration, should increase the chances of the user forming mental linkages between the various depicted concepts and their relationships (Holliday, 1976). It follows, that flow diagrams have been described to have sequential structure and therefore direction, afforded by the information that is depicted, along at least one coordinate and are therefore classified by Hardin (1993) as being ordinal or formal; and by Fry (1981) as complex lineal graphs that also have branching, feedback loops and diverse data.

In science, flow diagrams usually depict pathways or processes by reducing a sequential chain of verbal concepts (i.e. text) into a more manageable display (Holliday *et al.*, 1977). Since these diagrams are the primary tools for communicating process information, their interpretation and use, therefore, constitutes a student's understanding of the depicted information (Kindfield, 1992). For example, a flow diagram depicting an ecosystem is classified as having a functional role, whereby the user is able to follow a sequence of events that correlate to the organisation of a system (Duchastel & Waller, 1979). This account complements the definition of a flow diagram offered by Holliday *et al.* (1977) who says that without the flow diagram, a huge amount of text would be required to explain the ecosystem that is depicted. When examining the Concise Oxford Dictionary's meaning of flow chart/ diagram/ sheet: "diagram of movement or action of things or persons in a complex activity", it was evident that the dictionary took what was being depicted (a process or actions), as a criterion for classifying a flow diagram. This is, therefore, also the definition that we have adopted in classifying the diagrams used in the studies reported in this dissertation.

Now that we have clarified the term "diagram" in the context of the present study, we take a general look at the field of diagram research, of the effectiveness of diagrams in instruction and learning. Following this, a closer look at diagrams as visualisation tools will be considered in Section 2.3.

2.2 The Effectiveness of Diagrams in the Instruction and Learning of Science

“‘A picture is worth a thousand words’, aptly expresses the value of an image to aid our cognitive processing of information. Unfortunately we are often unable to write the thousand words that describe the image” (Lohse *et al.*, 1991). This quote suggests that although there are advantages of using diagrams in instruction and learning, students may have difficulty in interpreting and expressing their understanding of diagrams (as will become apparent Chapters 5- 7 of this dissertation). This section of the review therefore considers research that reports on the effectiveness of diagrams in the instruction and learning of science.

There are many reports on the uses of diagrams. The roles of diagrams in instruction include reinforcing and replacing text, summarizing, clarifying, grouping and comparing information (e.g. Schnotz, 1993, 1993b & Holliday, 1990) and illustrating abstract concepts by providing analogies (Bean *et al.*, 1990 & Hurt, 1987). Apart from these uses of diagrams in an instructional role, they also, have facilitative effects on student learning and understanding at both cognitive and motivational levels. An example of a motivational effect, includes increasing students’ attention and aiding poor readers; while cognitive effects, include aiding integration of new knowledge into existing knowledge structures, supporting cognitive processing and retention, and facilitating and encouraging problem solving (e.g. Schnotz, 1993, 1993b, Holliday, 1990, Levie & Lentz, 1982 & Dwyer, 1969). In contrast to the facilitative effects of diagrams, Holliday and Harvey (1976) mentioned that there are, of course, disadvantages of placing diagrams in text. For example, diagrams can divert or distract the reader’s attention away from the text. In addition, misleading or inaccurate diagrams can also hinder the students’ learning of scientific concepts. Through their experiments, Glenberg and Langston (1992) argued that diagrams do not offer the reported retentional and motivational characteristics that are attributed to the facilitative effect of pictures and text. This statement sets the tone for this section of the literature review as, throughout this section, we consider both the advantages and disadvantages of diagrams in the instruction and learning of science.

With respect to explicative diagrams, Duchastel and Waller (1979) identified seven major roles of diagrams, and stated that a particular diagram may show more than one of these namely, descriptive (give an over-view), expressive (make an impact), constructional (showing how a system fits together), functional, logico-mathematical (e.g. graphs), algorithmic (showing possibilities) and data display (e.g. for quick visual comparisons). While some diagrams serve a secondary or motivational role (e.g. attracting the reader's attention or repeating extracts from the text); other diagrams, such as graphs and charts, have the primary function of conveying information (Lowe, 1991).

In the context of biochemistry, diagrams have the instructive role of conveying information, as they are used as tools for communicating information about biochemical processes. One only has to page through a biochemistry or biology textbook to appreciate the variety and extent to which diagrams are used. Flow diagrams are typically used to depict biochemical processes and the understanding of these processes is central to our understanding of the function of living organisms. It follows therefore, as Kindfield (1993/1994) has suggested, that diagrammatic representations and diagram skills are vital components of what is learnt about biological/ biochemical processes. Chapters 5- 7, are concerned with the role of diagrams in student understanding of biochemical processes and whether these diagrams affect students' visualisation of biochemical processes, which is discussed in detail in Section 2.3. The next section considers some of the more general literature on the effectiveness of diagrams in instruction and learning.

2.2.1 Visiospatial Relations that Support Perception and Search in Diagrams

It is sometimes difficult to express the spatial information displayed in diagrams with words (Levie & Lentz, 1982). Diagrams help resolve this problem as they can save space and often communicate concepts, better than words (Fry, 1981). Thus, our perceptual systems can organise detailed representations effectively (Glenberg & McDaniel, 1992). This may be the reason why diagrams or icons often replace text in many situations, such as on computers and microwaves and even in education. Often, an extensive number of words are required to communicate the spatial relations conveyed by diagrams. This introduces various cognitive constraints such as learnability and communication speed (Glenberg & McDaniel, 1992). Thus, one of the instructional advantages of diagrams is believed to stem from their ability to show spatial relations between the concepts and the

objects that they illustrate (Winn, 1991b, Winn & Holliday, 1981 & Larkin & Simon, 1987).

In this regard, various authors have shown that, in contrast to textual representations, diagrams decrease the amount of search, and thereby increase their computational advantage when individuals are required to identify and extract information. Larkin and Simon (1987) support this in their seminal paper, "Why a diagram is (sometimes) worth ten thousand words", and also attribute the effectiveness of diagrams in problem solving to their computational advantages. In agreement, Winn (1993) has reported that in the case of diagrams where conceptual relationships are expressed spatially, i.e. where related concepts are placed in close proximity to each other, problem solving by graduate students was more rapid than with text. In addition, students who were familiar with the terms and conventions of the content were more rapid problem solvers (Winn, 1993).

The computational advantages of diagrams have been attributed to the fact that diagrams support humans' perceptual systems. In this regard, diagram elements and information is often grouped according to location and symbolic labels, which allows for perceptual inferences, which are natural for humans (Larkin and Simon, 1987). In light of this, Larkin and Simon (1987) have suggested that search in diagrams is less laborious than in text because once the first piece of information has been located, the next will be in an adjacent position. Also, the relationships between diagram elements are displayed explicitly and can be perceptually distinguished as either direct or indirect, thus allowing for the identification of selected information in reasoning applications (Pearl, 1992). In addition, Szlichcinski (1979) attributes the computational advantages of diagrams to the manner in which information is represented; as a simplified or organised form that allows for easy mental transformations during problem solving. In order for the reported computational advantage of diagrams to be realised, Larkin and Simon (1987) caution that an individual will have to be familiar with the appropriate computational processes in order to successfully problem solve with a diagram. The above brings us to the difficulties diagram users may encounter when interpreting or reading a diagram.

In terms of reading diagrams, diagram users often interpret diagrams in a random fashion (Lowe, 1997 & 1993). This is because guidelines for diagram interpretation are seldom provided since diagrams are often believed to be self-explanatory, even though they do not

always follow conventional reading strategies, which may prove to be problematic in abstract representations (Lowe, 1997 & 1993). An example of where this unconventional reading strategy may prove problematic was provided by Stylianidou *et al.* (2002) who found that mixing horizontal and vertical layout patterns within a diagram, caused diagram difficulties in a document that also contained text. This finding supports a suggestion by Winn and Solomon (1993) that conventional reading sequences should be employed in diagram design. With regard to flow diagrams, Hardin (1993) states that the rules for design are less explicit than those of maps, drawings and graphs, and a violation of the rules of flow diagram design, might for example, be an element being placed out of sequence, or when linkages infer untrue connections between elements. Therefore, to aid the user in the reading of flow diagrams, Jansen and Steehouder (1996) state that instructions (e.g. for a task) should be presented in the order they are carried out. In this regard, they propose two methods for flow chart designers based on the “average least effort” and “selection” principles, that allow the designer to determine which order of instructions is most effective (Jansen & Steehouder, 1996).

As a summary of the above, Arnheim (1970, pg 309), states that a diagram should be designed to favour perception or else it will not convey propositions perceptually and will be confusing and useless. As a result of disobeying the rules of visual perception (Arnheim, 1970, pg 309), visiospatial characteristics will not always represent the functional and conceptual relationships between the diagram elements. Therefore, students may not interpret the diagram as the author intended (Lowe, 1997 & 1993). In support of the above, Leonardo da Vinci is believed to be so successful in his anatomical drawings because he used spatial relations to show functional connections (Arnheim, 1970, pg 313), by following the rules of perception.

Apart from a diagram being visiospatially designed to promote perceptual inferences, the characteristics of the diagram user should also be taken into account. It has been reported by various authors (e.g. Stylianidou *et al.*, 2002 & Lowe, 2000) that, if students lack the context-specific knowledge about the depicted situation, they will use their prior knowledge to make sense of a representation, resulting in an interpretation that is not scientifically acceptable. For example, Lowe (1994 & 1994b) found that novices who lacked content knowledge, drew diagram elements inconsistently, as they missed the subtle information represented by visiospatial characteristics. Thus, these students characterised

diagrams based on their visiospatial characteristics rather than their semantics (Lowe, 1993). Although perception plays a major role in diagram interpretation, the skills required for effective diagram use need to be learned (Cheng *et al.*, 2001). Additionally, if students are not “visually literate” they may have difficulty in extracting information from static as well as dynamic visuals (e.g. Stern *et al.*, 2003). The important role of visual literacy and content knowledge in diagram use is discussed in greater detail in Sections 2.3.1 and 2.3.3.3 respectively.

Now that this section has highlighted the importance of visiospatial characteristics in search and interpretation of diagrams, I consider diagram effectiveness in terms of selectivity and abstraction in diagram design.

2.2.2 Selectivity and Abstraction in Diagram Design

“Graphical representations share a problem with textual ones: quality is not guaranteed. There are bad diagrams as well as good ones, just as there are both bad and good textual representations” (Petre & Green, 1993). But, what constitutes a good from a bad diagram, and how can we ensure that students are learning from good diagrams? With the assumption that the student will interpret the diagram as the author intended, a good or effective diagram is one that is used easily by human beings (Kosslyn, 1989). Also, it is one that requires a small amount of information processing to transform the symbolic information (MacGregor & Slovic, 1986). Therefore, this section briefly considers the literature on diagram design that specifically pertains to the selectivity and abstraction of diagrams and how this affects students’ interpretation and use of diagrams. Suggestions for diagram design are readdressed in the appropriate chapters and in Chapter 8 of this dissertation.

It is thought that many diagram designers base the choice of illustration on their own intuition, since there is no conceptual vocabulary or rationale, for guiding them in the illustration of instructional texts (Duchastel & Waller, 1979). The diagram designer assumes the diagram user is familiar with, and aware of, certain aspects of the diagram such as content and diagrammatic conventions that are used. Also, it is assumed that the viewer realises that diagram elements may have been abstracted, generalised and selectively represented (Lowe, 2001, pers. comm.). In view of this, Pintó and Ametller (2002) believe that the diagram designer should make all the information that is required

for interpretation of the diagram, explicit to the diagram user. However, Lowe (1994) cautions that too much information might cause cognitive overload and should, therefore, be avoided where possible.

Highly selective diagrams, that depict certain elements and their relationships by omitting their wider context to give the diagram a sharp focus, are believed to have an advantage over text (Lowe, 1994). Examples of selective diagrams include biochemical processes (Fig. 6.1 & 7.1, Chapters 6 & 7 respectively), where a specific process is depicted in isolation from other biochemical processes, and the wider context of where it occurs is omitted. Selectivity in diagrams is achieved in many different ways. For example, omissions of certain diagrammatic elements, level of abstraction, reduction of diagram complexity, and the use of artistic features, are all associated to increasing the specificity of diagrams (e.g. Lowe, 1993). These omissions or inclusions are said to “heighten the senses” (Nersessian, 1992). In addition, diagram selectivity is also enhanced by determining the scope of the depiction and which elements are to be shown in the diagram (Lowe, 1994).

Although diagrams are often “selective”, the success of their representation is determined by a number of other factors. For example, Lowe (e.g. 1994 & 1993) states that it is challenging for novices to build mental models of a depicted system from abstract diagrams. Although well-designed diagrams are an effective means of portraying information, whether an individual is successful at interpreting and using the diagram, depends largely on the individual’s prior knowledge (e.g. Lowe, 1996). This is because a diagram does not always contain all the required information to allow for effective use (e.g. Cheng *et al.*, 2001). Implications for this are 2-fold. Firstly, the information required by the user needs to be accessible and the representation must successfully depict the information required by the user (Petre & Green, 1993). Secondly, the individual needs to possess the associated content knowledge (e.g. Lowe, 1994 & 1993) and has to be sufficiently visually literate (e.g. Pintó & Ametller, 2002). Visual literacy and content knowledge, both required by the user to successfully interpret a diagram, are readdressed in Sections 2.3.1 and 2.3.3.3, respectively.

In terms of flow diagrams used to represent biochemical processes (Figs. 6.1 & 7.1, Chapters 6 & 7 respectively), the highly abstracted nature of the diagrams is not meant to

be interpreted literally (Lowe, 2000). This high degree of abstraction, and the nature of the content depicted in flow diagrams, is more difficult to process than realistic pictures (Lowe, 1994). Additionally, although some diagrams help people perform tasks, these diagrams place a greater interpretive burden on the user (Szlichcinski, 1979). For example, in diagrams that depict biochemical processes the elements portrayed in the diagram often do not resemble what they look like in real life. Rather, the function of these types of diagrams is to depict the relationships between the elements (Lowe, 1986). Therefore, the omission of certain features for the purposes of adding clarity, and for the transformation of certain elements, can contribute to student difficulties with understanding these processes. This is because they still have no means through which to visualise the process realistically (Lowe, 1986).

As will be readdressed in Chapter 8 of the dissertation, there are many other features of the diagram that may cause confusion for diagram users, such as the spatial arrangement and ambiguous use of arrows (e.g. du Plessis *et al.*, 2002 & Stylianidou *et al.*, 2002), as well as the use of colour. As an example, colour has been shown to restrict the scanning of a diagram and directs the user's attention to important parts of the diagram (Holliday, 1990 & Reid & Miller, 1980). A lack of highlighting has also been shown to cause difficulty when interpreting diagrams (Stylianidou *et al.*, 2002). In contrast, colour has also been found to be a distracter, drawing attention to less important features of the diagram (Reid & Miller, 1980). In this regard, colour does not necessarily motivate or change students attitudes to science, but rather provides an attractive and marketable product for publishers (Holliday, 1990). Therefore, although colour has been reported to improve student achievement at certain educational levels (e.g. Dwyer, 1970), Paivio *et al.* (1968) have stated that the effectiveness of colour in a diagram does not render the coloured diagram to be superior to one without colour. Thus, this mixed response to the use of colour by various authors indicates that students' ability, the type of diagram, and depicted content, all influence whether colour can aid student interpretation of the diagram. This reiterates the importance of linking diagram design studies with studies of the diagram users themselves, as well as the content that is represented by the diagram.

2.2.3 Information Retention and Recall From Diagrams

Levie and Lentz (1982) reviewed a variety of studies on the effects of pictures and diagrams on learning when combined with text. They concluded that the presence of pictures allows better memory of information under certain conditions (Levie & Lentz, 1982). The advantage of diagrams in the memorisation and recall of information, when combined with text in instruction, has been attributed to an elaboration of the dual coding theory of Mayer (e.g. 2003 & 1997). As pointed out, according to this theory (Chapter 1 & Chapter 8, Fig. 8.1) two information-processing systems are believed to operate: a verbal and a pictorial system (Mayer, 2003 & 1997). Referential connections are made between the visual and verbal representation systems and between the learners' prior knowledge (Mayer, 2003 & 1997) during diagram interpretation. It has, therefore, been suggested that dual-coding of information is advantageous over single-coding (Lewalter, 2003, Mayer, 2003 & Schnotz & Bannert, 2003). With this in mind, some studies that have shown increased memory and recall of information through the use of diagrams will now be considered.

Representational pictures or diagrams have been shown to facilitate learning as they aid recall, but this is not always the case, especially with content that is more abstract or complex (Alesandrini, 1984). Learners who are able to process the information in abstract diagrams (e.g. flow diagrams) when combined with text may learn more, even though the use of these diagrams has been shown to be problematic (Levie & Lentz, 1982). Egan and Schwartz (1979) showed that skilled electronics technicians (experts) were able to recall more of a circuit diagram after brief exposure than novices. They also reported that experts, in contrast to the novices, were able to build circuits from memory through the "chunking" of functional units, where the size of the expert chunks increased with prolonged exposure to the diagram (Egan & Schwartz, 1979). The results of this study also support similar claims made by other authors (e.g. Lowe, 1996 & 1994) that a certain amount of conceptual knowledge is required for effective use of a diagram (see Section 2.3.3.3).

Dwyer (1969, 1968, 1967) has also measured retention and recall of information in various studies using 3 diagrams of the heart that varied in their degree of abstraction (from photograph to shaded diagram to line drawing). In one of the studies conducted with 86 freshmen students, Dwyer (1967) showed that the photographic representation was the

most effective in promoting an integrated understanding of the presented concepts, locations and structures of parts of the heart, and in promoting effective transfer of learning. A subsequent study using the same 3 diagrams with 129 9th-grade students revealed that, in conjunction with the above findings on the photographic representation, the abstract line diagram was more effective than the shaded diagram shown in a delayed retention test (Dwyer, 1968). Although Dwyer (1969, 1968, 1967) and Reid (1990) have suggested many reasons for the general effectiveness of different types of diagrams, Dwyer (1969 & 1967) has concluded that not all visual representations were equally effective for meeting their educational objectives. This suggests that the effectiveness of diagrams during memory and recall of information depends on a number of factors including student ability, type of diagram, information content of text and diagram, and what was being tested for, i.e. the recall and not the understanding of information. In an extension of this section, the following section considers the cognitive effects of diagrams on learning.

2.2.4 Comprehension and Understanding Using Diagrams

Diagrams may free or expand the functional capacity of the working memory, thereby improving comprehension (Glenberg & Kruley, 1992). Combining diagrams with expository text (where factual knowledge is learnt) is believed to be superior to text-only forms of information (Lewalter, 2003). This initial evidence was provided by Holliday (1975), who combined simple line drawings with verbal labels and descriptions of the drawings, and found that the verbal comprehension of tenth grade biology students improved significantly. Mayer (2003) found that students were found to learn more deeply from pictures and words combined (“multi-media effect”), than from words alone. In terms of static diagrams versus animations, the use of static visuals was superior over animations due to animations placing different cognitive demands on the user (Lewalter, 2003). This issue is discussed further in Section 2.3.2.2.

The role of diagrams in student understanding has also been reported by a number of authors. An example are those of conceptual models, described as either words or diagrams that aid learners in mental model construction by highlighting important information, which have been reported to aid learners’ understanding (Mayer, 1989). In one study, Korean high school students’ conceptual understanding at the molecular level, was believed to be more emphasised through the use of diagrams (Noh & Scharmann, 1997). These students were found to construct more scientifically correct conceptions than

students who received traditional instruction on introductory chemistry concepts (Noh & Scharmann, 1997). However, no facilitative effect was seen in the problem solving ability of the students, possibly due to the students not receiving any training on problem solving, their memorization of formulae, and their attitude and interest in chemistry (Noh & Scharmann, 1997). In addition, promoting conceptual change (by challenging a number of commonly held misconceptions) and aiding problem solving through the development of novel diagrams depicting the properties of the electric circuit, has been shown by Cheng and Shipstone (2003).

Thus far, Section 2.2 has presented conflicting evidence and views on whether or not diagrams are effective teaching and learning tools. I summarise the literature viewpoints in the following section.

2.2.5 Summary and Conclusions

To investigate whether a diagram is effective in combination with text in instruction and learning, a researcher would have to ensure that the students in the sample are in fact using the diagram. Evidence for this has been provided by Holliday *et al.* (1977 & Holliday, 1976) who reported that students tend to exert the least amount of effort required to perform a task, and therefore suggested that a learner would tend to use a more familiar medium (which was text in 1977) when provided with a task, thus neglecting the less familiar medium (i.e. a diagram). Levie and Lentz (1982) also drew attention to this and concluded that diagrams are useful, as long as the learners process them (which may involve prompting if they are unaware of how to make effective use of them). A method for ensuring that learners use the diagram in these types of studies, was achieved by Holliday *et al.* (1977 & Holliday, 1976) who placed instructive questions directly below the diagram in his study, in an attempt to persuade subjects to consult the diagram when answering the questions. To circumvent the concern that students may not be using the diagram, the studies reported on in this dissertation (Chapters 5- 7) investigated students' understanding of the diagram in isolation from the text (apart from the appropriate diagram caption), which ensured students were extracting information from the diagram. In this regard, Lowe (1993) has stressed the importance of investigating diagrams in isolation from text, since they are often the primary tools for conveying information.

During a review of the literature, it has been evident that many authors have provided a range of evidence either in support for or against the use of diagrams in the instruction and learning of science as discussed in the above section (2.2). Overall, the effectiveness of diagrams has been related to a number of factors. For example, different visuals (e.g. graphs, animations) differ in their effectiveness according to the type of information being communicated (e.g. Lohse *et al.*, 1991 & Dwyer, 1970). Thus, the effectiveness of a diagram depends on the type of information required by the student (e.g. Lohse *et al.*, 1991 & Dwyer, 1970). When evaluating the effectiveness of diagrams, factors such as what is being measured (e.g. recall or problem solving) and the ability of the students themselves (Winn & Sutherland, 1989) should also be taken into account. This is because by changing these two variables, different conclusions on diagram effectiveness in instruction and learning may be drawn. In accordance with this, we now consider these variables in more detail.

Many authors have illustrated that different types of visuals differ in their effectiveness (e.g. Lohse *et al.*, 1991 & Dwyer, 1970). For example, Bean *et al.* (1990) have supported the use of analogical pictures, where for instance the cell might be represented as a factory when instructing high school biology students. In another account, Hurt (1987) has shown that, although analogical diagrams are an effective means for depicting abstract concepts (e.g. muscle contraction) to college students, representational pictures are more effective for depicting information about phenomenon. In flow diagram research, Holliday (1976) found that both a picture-word and a block-word flow diagram were more effective than stand alone text. However, neither was more effective when combined with text when compared to text that stands alone (Holliday, 1976). Holliday's (1976) results were limited to the teaching of sequences of verbal concepts where instructive questions were included. He also stated that his study did not prove that flow diagrams were better than text, as many variables exist such as the information content and the arrangement of verbal (text) and non-verbal (diagrams) stimuli, as well as student learning behaviours (Holliday, 1976). This finding brings us to the next factor in assessing the effectiveness of diagrams, i.e. the student's ability.

Returning to the point made at the beginning of this section (that researchers and instructors should ensure students are in fact using the diagrams presented to them), an interesting study by Dwyer (1969) evaluated whether the diagrams that 86 university

students thought were effective, corresponded to their actual achievement. This study revealed that the diagrams students thought were most effective, were not necessarily those that were most effective in terms of their achievement as measured by 5 criterion tests. This indicated that the ability of the students played a major role in their success with interacting with the diagram, even though they may have favoured a particular diagram. In terms of student ability and type of diagram, a study with high school introductory biology students by Holliday *et al.* (1977) revealed that low verbal performers given a picture-word flow diagram performed better than the low verbal performers who were given the block-word flow diagram. He also found that the higher verbal ability students performed better with the block-word diagram than did the lower ability verbal performers (Holliday *et al.*, 1977). Similarly, another study by Winn and Sutherland (1989) revealed that low-ability students recalled more when they were presented with icons than when presented with labelled squares. However, no difference was seen with the higher ability students in this study, which tested students' ability to recall names and locations of elements in maps and diagrams (Winn & Sutherland, 1989). In addition, the evaluation of recall in the latter experiments indicated a facilitative effect offered by the use of diagrams, but when cognitive processes such as pattern encoding were investigated, the use of labelled squares was found to be superior to icons (Winn & Sutherland, 1989). Thus, this latter finding illustrates that, by changing what is being measured when assessing the effectiveness of diagrams, the researchers' interpretation on whether or not a particular visual is effective at communicating a particular type of information may change.

The discussion above suggests that due to the large number of variables and both positive and negative reports, the effectiveness of diagrams in teaching and learning of science is subjective. Therefore, studies need to take various factors into account such as the age and ability of students, what is being tested for (e.g. recall), the type of diagram effective for the type of information, and whether multimedia is to be used (e.g. text and diagram), before conclusions or assumptions can be drawn. Since the studies reported in this dissertation focus on diagrams as primary visualisation tools of biochemical processes, the next section of this review focuses on various factors that affect students' visualisation of the information depicted in diagrams.

2.3 Diagrams as Visualisation Tools

The above section of this review (2.2) concentrated on the general effectiveness of diagrams, particularly when combined with text, in the instruction and learning of science. The following section focuses specifically on diagrams as visualisation tools, by considering associated topics such as visual literacy, visualisation and mental models.

2.3.1 Visual Literacy

In the past, a literate person was considered to be someone able to read and write (Seels, 1994). However, today's literate person is considered an individual able to show both verbal and visual literacy skills (Seels, 1994). Therefore, in order to achieve *full* literacy, students should be encouraged to analyse and use different forms of representation of information such as texts and diagrams in different contexts of science (e.g. Roth, 2002). Visual literacy per sé has been described as the ability to read, interpret and understand diagrams/ images, in order to learn, think and construct scientific diagrams and thus communicate ideas (Lowe, 2000 & 1987; Braden & Horton, 1982 & Fry, 1981). Thus, visual literacy has been described by Seels (1994) as a concept aided in definition by the constructs of visual thinking and communication.

Verbal literacy is developed from an early age by formal instruction. This is often not the case with diagrams and other visuals. Although diagrams are often used as the primary teaching tools in science, visual literacy is given little or no attention (e.g. Lowe, 2001, pers. comm.). A deficiency in visual literacy would not be noticed easily as it would in terms of text (Lowe, 2001, pers. comm.). However, this visual literacy deficiency could lead students not to grasp more complicated scientific concepts and the relationships between them (Lowe, 1987). Thus, Ametller and Pintó (2002) state that visual literacy should be expected of all citizens and should therefore be taught, while Sigel (1978, as cited by Lowe, 1991), views visual literacy as a learned skill that develops over time. It follows, that often during diagram learning, little attention is paid to diagrams, as some students do not know how to process them effectively (Schnotz, 1993). In this respect, sometimes learners have been found to consult text before diagrams as they believe more can be learnt from the more familiar medium, due to the amount of school time that is

devoted to it (Holliday, 1976). Since diagrams are the primary tools for communication of information in many disciplines (e.g. biochemical processes), and the fact that many students do not use diagrams effectively, the need to teach visual literacy to students is clearly of great importance. This brings us to the question of when to begin visual literacy instruction. Fry (1981) believes there is a need to teach visual literacy throughout an individuals' education with most emphasis in the upper elementary and middle schools, while Lowe (2000) believes visual literacy instruction should begin before the student begins formal science and technology instruction. The subsequent question of "what to teach" in terms of visual literacy would be governed by what is required by a diagram user to make effective use of the diagram. Adequate reading of a diagram requires the user to decide on the context, meaning of the elements within the diagram, and the relationships between the diagram elements (Hill, 1988). Also, since the transformations and conventions used in diagrams are not those seen in everyday life, the user needs to be taught how to deal with these in order to interpret the diagram as was intended by the author (Lowe, 1993b). Therefore, what a reader sees in a diagram is what the reader has learnt to look for, and the skills necessary for reading graphical representations should not be underestimated (Petre & Green, 1993).

Coupled to the above discussion is the construct of "visualisation", the success of which would be determined by, among other factors, whether an individual is visually literate. We now consider the idea of visualisation in science, as this is a major focus of this dissertation.

2.3.2 Visualisation

The following subsections, consider the meaning of the widely used term "visualisation" in relation to the studies reported in this dissertation (Section 2.3.2.1), the range and development of visualisation tools (Section 2.3.2.2) and makes mention of some of the visualisation difficulties that have been identified in various scientific disciplines (Section 2.3.2.3).

2.3.2.1 What is "Visualisation"?

The word "visualisation" has different meanings depending on the context. The everyday meaning is to "see a mental image" (Sanders, 2003, pers. comm.) although "visualisation"

is often used loosely in literature (Anderson, 2003, pers. comm.). Visualisation as a research interest is becoming increasingly popular in diverse fields. For example, Cawkell (2001) has stated that the use of the term “visualisation” in article titles found on the “Web of Science” database, increased from 32 in 1955, to 2771 in 2000. Although this may be attributed to databases only recently listing and updating by adding articles on visualisation, the current interest in “visualisation” is a rapidly growing field with many applications in all spheres. For example, in the design of CD_ROMs (e.g. Multimedia Science School, www.curriculumonline.gov.uk, for chemistry, biology and physics teaching materials), and in research into the effectiveness of multi-media, computer-based animations, simulations (e.g. Mayer, 2003 & Yair *et al.*, 2003) and databases (Song, 2000 & Cawkell, 2001). All of the above are designed for the purpose of aiding visualisation, understanding and information retrieval. The development of instruments to measure and isolate visual processing and understanding (e.g. Cataloglu & Robinett, 2002 & Van Dusen *et al.*, 1999), is another extension of the current field of visualisation. Despite all this current research, there is no set definition for the term “visualisation” that can be used in a science education context. However, we will attempt to define this term in the context of this dissertation.

Schnotz (2003, pers. comm.) views “visualisation” as the process whereby a picture or image is formed from information that has already been understood and is stored in memory, i.e. a mental model or a propositional representation has already been constructed as a prerequisite to the process of visualisation. In contrast, McCormick *et al.* (1987) view visualisation more broadly as being the perception, interpretation, use and communication of visual information, thereby encompassing the entire process from reading a diagram to externally expressing an interpretation of it. In line with the definition proposed by McCormick *et al.* (1987), Anderson (2003, pers. comm.) views visualisation as a reasoning process involving the visual display (e.g. diagram) and the diagram-relevant conceptual knowledge of the user. Thus, he describes visualisation as “the development, and use of mental models of objects, processes and concepts. The use of the mental model will involve its expression and application to the generation of new ideas, information, predictions and solving problems” (Anderson, 2003, pers. comm.). This, therefore, reiterates the view of Franco *et al.* (1999) who state that mental models involve visualisation as it supports scientific thinking and the use and development of mental models.

In the studies reported on in this dissertation, we adopt the above interpretation of the term “visualisation” as stated by Anderson (2003, pers. comm.). This interpretation will be revealed through our research process, where students’ understanding and visualisation of biochemical processes were investigated through their interaction with a variety of diagrams, and the subsequent expression of their understanding (Chapters 5- 7). As a result of our research process and the analysis of our data, we shall propose a preliminary model of visualisation in Chapter 8. In biochemistry, diagrams serve as visualisation tools, the development and range of which, are discussed in the following section.

2.3.2.2 Visualisation Tools

Visualisation is aided and expanded through the development and use of visualisation tools such as interactive video, photography, computer graphics, 2D diagrams and physical models. These visualization tools are important in abstract contexts, e.g. biochemistry, as the concepts involved are difficult to view directly (Anderson, 2003, pers. comm. & Schönborn *et al.*, 2003). The evolution of visualisation tools is illustrated, for example, by the development over 20 years ago of ribbon-structures used to translate the 3-D structure of proteins into static 2-D representations on paper, to the current development of movies, physical models and more recently interactive graphics such as Kinemage® by the same authors/artists (Richardson & Richardson, 2002).

Evidence supporting the use of visualisation tools in the instruction and learning of science, has been provided by a number of authors. For example, static diagrams acting as additional electrochemistry visualisation tools had a positive effect on learning of secondary school chemistry students (Brandt *et al.*, 2001). Furthermore, support for more dynamic visualisation tools was shown when secondary school students, who showed difficulties with visualising molecular rotations in static diagrams, were found to show significant learning when multicoloured slides were presented simultaneously (Shubber & Al-Mudaifa, 1991). Moreover, varied degrees of support are shown for some of the more dynamic visualisation tools available (e.g. multimedia on the web (Agapova *et al.*, 2002) and CD ROMs that accompany some of the latest biology and biochemistry textbooks), which are currently being developed and tested. In this regard, although simulations (interactive animations) have been reported to improve student understanding of biological processes, static diagrams were found to be superior over animations for aiding students’

comprehension of these processes (e.g. Nerdel *et al.*, 2002). Current investigations into the use of animations, over still or static diagrams, as visualisation tools are currently under way and being reported on by a number of leading researchers in the field, as outlined below.

Animations as visualisation tools are believed to eliminate any misunderstandings that may arise from students misinterpreting static diagrams. For example, arrows in static diagrams represent movement, but there is no need for arrows in animations as this movement is shown. This is believed to free working memory for cognitive processes (Nerdel *et al.*, 2002), and eliminate any possible misinterpretations. Similarly, Nerdel *et al.* (2002) and Lewalter (2003) also believe in the power of animations as aids for students' visualising spatial aspects of dynamic and complex biological processes, thereby allowing students to construct adequate mental representations. Despite the power of animation, Lewalter (2003) suggests that static visuals can be superior to animations as the learner controls the speed of their learning and construct their own visual models of the dynamic process. However, interactive graphics do allow the learner to work at their own pace (e.g. Richardson & Richardson, 2002) and, at the same time, also provide the power of dynamic animations as visualisation tools. There is, however, the challenge of interactive graphics to successfully guide the user through cues to perceive the key features (Richardson & Richardson, 2002) as perceptually salient objects are given more attention through the use of colour or movement (Lowe, 2003). Other disadvantages of multi-media visualisation tools are that movies are effective but not easily modified, physical models are expensive and limited in what they may depict, and there are limiting features of the learner such as prior knowledge (see Section 2.3.3.3) and cognitive overload (Nerdel *et al.*, 2002). In terms of the superiority of static diagrams versus animations as visualisation tools, the field of research into multi-media based instruction is still too new to make any concrete assumptions.

With the increase in the number and range of visualisation tools in education, their effectiveness in instruction is of great importance. This has been realised by Cataloglu and Robinett (2002) who have developed an assessment instrument, in the form of a survey, to probe students' conceptual and visualisation understanding in quantum mechanics; and van Dusen *et al.* (1999) who have developed a tool that isolates visual processing. There has also been research into students' visualisation difficulties in general, and with respect to the role of diagrams as visualisation tools, as discussed in the following section.

2.3.2.3 Visualisation Difficulties

The large body of literature that encompasses students' conceptual and reasoning difficulties in various disciplines may also, in many cases, be considered to be representative of students' visualisation difficulties. This is based on the meaning we assign to "visualisation" as discussed earlier in Section 2.3.2.1. Thus, student conceptual and reasoning difficulties in the content areas of physics (e.g. Harrison *et al.*, 1999), chemistry (e.g. Garnett *et al.*, 1995) and biology (e.g. Marek, 1986) have been well researched and reported on, but the field of biochemistry is less well researched (e.g. Anderson *et al.*, 1999 & Fisher, 1985).

In terms of the visualisation of diagrams, a model by Schönborn *et al.* (2003 & 2002) has been used to investigate 3 factors that affect students' interpretation of diagrams namely, their prior or conceptual knowledge (see Section 2.3.3.3), their reasoning ability (see Section 2.3.3.4) and the affect of the diagram itself (see Sections 2.2.1 and 2.3.3.2). This model (Schönborn *et al.*, (2003 & 2002) is discussed in more detail in Chapter 3 (Section 3.3.8) and applied to the results in Chapters 6 (Section 6.3.4) and 7 (Section 6.3.3). There is literature on students' visualisation difficulties with diagrams that may be attributed to any one or combinations of the factors investigated by Schönborn *et al.* (2003 & 2002). For example, students difficulties with the visualisation of IgG (immunoglobulin G) molecules (Schönborn *et al.*, 2003 & 2002); difficulties with the visualisation of processes depicted by symbolic conventions such as arrows (du Plessis *et al.*, 2002 & Hull *et al.*, 2002), difficulties with the spatial visualisation of biomolecules (Makura *et al.*, 2000) and biological sections (Sanders, 1995), and difficulties with the visualisation of macro-micro symbolism in chemistry (Johnstone, 1991). Literature dealing with visualisation difficulties in various disciplines and content areas will be revisited in the Chapters 5- 7 of this dissertation.

Section 2.3.2 dealt with the process of "visualisation" and made mention of mental models and the role of students' conceptual knowledge. The following sections consider mental models (Section 2.3.3) and diagram use (Section 2.3.3.2) as a function of visualisation. The role of conceptual knowledge (Section 2.3.3.3) and reasoning (Section 2.3.3.4) in mental model construction will also be considered.

2.3.3 What are Mental Models?

Mental models have been defined as internal representations constructed in the minds of individuals (Winn, 1991). Thus, they are representations of external systems and represent how individuals understand and interpret the world (Winn, 1991). They are also generative and have the capacity to deal with changes, thereby allowing inferences and new information of unknown situations to be formulated (Lowe, 2001, pers. comm. & Franco *et al.*, 1999). Johnson-Laird (1983, pg 23) describes inferences as the thought processes moving from one set of propositions to the next, whereby one conclusion is formed from either one, or many locations. Other properties of mental models are that they involve tacit (unspoken or implicit) knowledge, are synthetic, incomplete and constantly evolving (Kearsly, 1994- 2003). In addition they may contain errors since they are simplified representations that are constrained, not only by worldviews and general beliefs of people, but also by the limitations of the human information processing system (e.g. Franco *et al.*, 1999).

One of the applications of mental models, in terms of the inferences that are possible, are their use in problem solving. In order for successful problem solving and understanding to occur, the learner needs to have constructed an effective and useable mental model of the presented information, whether it is textual or diagrammatic (Lowe, 1996). For example, successful problem solvers in organic chemistry were found to be able to switch between representation systems, because their mental models contained elements of more than one representation system (Bodner & Domin, 2000). In addition, their internal representations were highly symbolic (e.g. equations), while less successful problem solvers constructed verbal representations (Bodner & Domin, 2000). Both internal and external representations are used during problem solving (Larkin & Simon, 1987) although there has been debate among authors on the nature of these internal representations that are constructed, i.e. whether they are propositional or diagrammatic. In view of this statement, the debate on the nature of internal mental representations and the construction of mental models will now be considered.

Typically, in order for an individual to construct a mental model, learnt facts in the form of pictures or words, should be organised and then integrated into conceptual frameworks based on the individuals' evolving prior knowledge (Anderson, 2003, pers. comm. & Ward

& Wandersee, 2002). Mental models integrate two types of information, the propositional information from the text and the descriptive perceptual information from the diagram (Glenberg & Langston, 1992). The organization of these 2 forms of information (pictures or words) into coherent representations, and then combining and linking them with each other and with the learner's prior knowledge in an iterative fashion, implies that mental model construction is a form of active learning, that can be described by the dual coding theory (Mayer, 2003). Referential links to prior knowledge render the mental model more information rich when compared to a visual perception, although mental models are more abstract and contain less irrelevant information than the perceptual image (Schnotz & Bannert, 2003). In contrast with the dual coding theory, Schnotz and Bannert (2003) view the mental model, derived from a diagram, to be separate from a propositional representation, which is derived from text. Nevertheless, these 2 cognitive modules interact in both text and diagram comprehension (Schnotz & Bannert, 2003). This brings us to a contentious issue termed the "imagery debate" that considers the nature of images in the mind.

2.3.3.1 The Imagery Debate

Johnson-Laird (1983, pg 146-147) argues that there are at least three forms of internal representation namely, mental models, propositional representations and images, and that the "imagery debate" is not about imagery, but about the nature of images as mental representations, as images cannot literally be 'pictures in the head'. The "imagery debate" involves two schools of thought about the nature of images as mental representations i.e. how information is actually stored in memory: as images or as propositions. The one school of thought involves the propositionalists (e.g. Baylor, Palmer & Pylyshyn) who view images to be constructed from propositions that do not correspond to words or pictures, as a form of making stored information easy to manipulate (Johnson-Laird, 1983). By contrast, the second school of thought involves the imagists (e.g. Paivio, Shepard & Kosslyn) who view images as being a type of mental representation that represents the object (Johnson-Laird, 1983). Regardless of whether the propositionalists or the imagists are correct, and regardless of *how* these images are stored, Anderson (2003, pers. comm.) states that "humans can 'see' or 'visualise' things with the 'mind's eye' and use this information to understand concepts, processes, and systems, express themselves, and solve problems". In this regard, this dissertation (Chapters 5- 7) is not concerned with *how* students' store information about biochemical processes or the nature of their images as

mental representations. Rather, we are interested in studying students' understanding or visualisation of these processes and identifying any visualisation difficulties students may have. Thus, we shall now move away from the imagery debate and deal with the effects and role of diagrams in mental model construction.

2.3.3.2 Diagrams and Mental Model Construction

The cognitive processes whereby mental models are constructed and used still render unsolved questions (Morra, 2001). Mental models are constructed through interactions between information stored in the long-term memory and information in the working memory of what we gather from the world around us (Lowe, 2001, pers. comm.). This explains why mental model construction is believed to be constrained by the human information processing system (Morra, 2001). Lowe (2001, pers. comm.) states that an external representation (e.g. diagram) reflects, or is an outcome of, the authors' mental model of the depicted content and is therefore characterised by the content and the purpose of the diagram, the transformations within the diagram and the assumptions that are drawn about the diagram user. It follows, that in order for a student to form an effective and scientifically acceptable mental model of the depicted content, the student would have to be visually literate (Pintó & Ametller, 2002), as was discussed earlier in Section 2.3.1.

The purpose of instruction is to aid students in constructing scientifically appropriate mental models of the situation being taught. In terms of dual coding theory, Mayer (2003) describes a cognitive theory of multimedia learning. He describes this theory as an iterative process whereby the learner selects aspects of the presented information to be further processed in their respective channels (i.e. verbal channel for words and visual channel for pictures), organizes the information into appropriate verbal and pictorial mental models and integrates their verbal models, pictorial models and prior knowledge (Mayer, 2003). Diagrams are believed to aid mental model construction as the spatial arrangement of diagram elements (see Section 2.2.1) allows for them to be integrated into the mental model in the same manner (Glenberg & Langston, 1992). This is in contrast to text, where the sequential nature of the information presented, requires more cognitive processing to accurately represent the situation (Glenberg & Langston, 1992).

Everyday cognitive schemata are used to perceptually interpret realistic diagrams such as photographs, but specific graphic cognitive schemata are required to interpret visiospatial

information from an abstract diagram (Schnotz & Bannert, 2003). In this regard, when constructing a mental model from a diagram, the diagram needs to be processed by the user at a number of levels. The first stage is perceptual processing whereby the diagram is classified at a visiospatial level, i.e. the elements making up the diagram are processed (see Section 2.2.1) (Schnotz & Bannert, 2003 & Humphries & Bruce, 1989). The second stage (semantic classification), involves relating the diagram elements to functional representations for the purposes of creating a propositional representation as well as a pictorial representation (Schnotz & Bannert, 2003 & Humphries & Bruce, 1989). Therefore, in order for a diagram to be effectively understood, a mental model based on semantic classification of the diagram would need to be constructed, by mapping diagram elements onto mental entities, and mapping spatial relationships onto semantic relations (Schnotz & Bannert, 2003). However, this might be challenging for novices who lack prior knowledge and may therefore build mental models based purely on the visiospatial characteristics of the diagram (Lowe, 1993).

Prior knowledge (see Section 2.3.3.3 that follows) also plays a role in the perception of the diagram, since learners with a low prior knowledge may be constrained due to cognitive overload (e.g. Goldman, 2003). Therefore, when constructing a mental model from a diagram, a certain amount of prior or content knowledge has the advantage that individuals will be able to make inferences from the diagram that would be connected to their background content knowledge (Lowe, 1993). In contrast, individuals with lesser content knowledge would only be able to form links between the visiospatial elements within the diagram (Lowe, 1993). Thus, it is important to integrate information from text, diagrams and prior knowledge as, acquiring information from a diagram may not be sufficient for students to achieve a comprehensive understanding of the topic (Reinking, 1986).

Apart from the role of prior knowledge in mental model construction from a diagram (discussed further in the following section), cognitive overload is another factor affecting how mental models are constructed from diagrams. In this instance, the development and construction of mental models may be restricted when viewing abstract diagrams that depict dynamic systems (e.g. biochemical processes). This is because the user would first have to process the spatial information that is used to represent a sequence of events or relationships between the depicted elements (Lowe, 2001, pers. comm. & 1996). The interpretation of the spatial information may be overcome with the use of more dynamic

visualisation tools, such as animations (although there are disadvantages associated with this type of instruction as discussed earlier in Section 2.3.2.2). Diagrams should, therefore, contain information in a form that does not require excessive cognitive recourses as the working memory can only deal with a certain amount of information at a given time (Lowe, 2001, pers. comm.). Thus, the information presented in the diagram should be able to be incorporated into the developing mental model directly (Lowe, 2001, pers. comm.).

Nevertheless, cognitive overload may be decreased through the use of multiple representations (Seufert, 2003). In this case, information is distributed over more than one representation. Following on from this, different representations are useful for different purposes, and one representation may aid in the interpretation of another representation (Seufert, 2003). In order to effectively use multiple representations and benefit from their constraining and complementary effects in relation to each other, learners are required to build referential connections between corresponding elements in the different representations (these comments bring us back to earlier comments about the importance of visual literacy in Section 2.3.1), and thereby construct coherent knowledge structures (Seufert, 2003). For example, chemistry experts were found to use many more types of representations (video segments, animations at molecular level, chemical equations and graphs) that were presented to them in order to group cards into meaningful sets that corresponded to the representations previously shown to them (Kozma, 2003). In contrast, novices used surface features of the representations to try and understand the chemical phenomena and had difficulty moving between different types of representation (Kozma, 2003). Therefore, experts were able to move between representation systems, identifying and linking features from the different representations to support and communicate the underlying principles that were represented (Kozma, 2003). The importance of conceptual knowledge has recurred throughout this literature review and we will therefore consider its role in terms of mental model construction.

2.3.3.3 Conceptual Knowledge and Mental Model Construction

This review has highlighted that an individual's lack of prior knowledge (e.g. Lowe, 1999 & Winn, 1993), their use of everyday knowledge and their lack of visual literacy (e.g. Pintó & Ametller, 2002 & Lowe, 1999 & 1996), can all have a negative effect on a diagram user's interpretation of a diagram. These effects have been reported by various

authors and may also be found to be applicable to the use of animations during instruction (e.g. Lowe, 2003).

Lowe (2001, pers. comm.) attributes a source of difficulties students may have with interpreting scientific diagrams, to the diagrams not containing all the necessary contextual information required to interpret them correctly. This may be due to clarity and economy of the diagram or because the user is assumed to be familiar with the conventions (Lowe, 2001, pers. comm.), which, in conjunction with diagram elements, may have a variety of meanings (Ametller & Pintó, 2002). For example, Ametller and Pintó (2002) found that identical arrows within an image were assigned different meanings largely based on the general context within which the student chose to interpret the image (Ametller & Pintó, 2002). As a result, people therefore “see” things differently because visual images are filtered (as per constructivist theory e.g. von Glasersfeld, 1992) through an individual’s unique prior or conceptual knowledge, which highlights the important role of conceptual knowledge in the visualisation process (Anderson, 2003, pers. comm.).

There are many advantages of prior or conceptual knowledge that a diagram user has for the interpretation of diagrams. For example, Winn (1993) has stated that diagram users with a broader content knowledge are able to search for, encode, process and recall the information more effectively than novices or less knowledgeable people. This is because perception organises the elements of a diagram irrespective of whether the user understands the content or not, and search of the diagram is governed by the user’s content knowledge of the subject matter and the symbols used in the diagram (Winn, 1993). In addition, conceptual knowledge may also overcome cognitive overload because as the number of relationships and elements that are depicted in the diagram increase, so do the cognitive processing demands (Lowe, 1999). Therefore diagrams illustrating dynamic situations may prove to be cognitively challenging for learners with a limited content knowledge (Lowe, 1999). In addition, some diagrams can be used to make inferences but whether or not this is successfully achieved, depends on the individual’s prior knowledge and their mental model of the diagram content (Cheng *et al.*, 2001). In support of this, Lowe (1994) found that experts were able to make inferences by using a diagram in conjunction with their prior knowledge whereas novices tended to be restricted to the visiospatial elements of the diagram (Lowe, 1996).

As I have shown, a level of content knowledge goes a long way in determining whether an individual will successfully use a diagram. In this respect, what types of knowledge are required? Lowe (1994 & 1993) describes two types of knowledge that the diagram user requires to effectively build a mental model from an abstract diagram: domain-general and domain-specific knowledge. The domain-specific knowledge is content specific information that allows the diagram user to construct a mental model involving the relationships between the elements of the diagram (Lowe, 1994 & 1993). In this respect, a high level of this type of knowledge has been found to aid in the processing of new information into related domains (Lowe, 1993) e.g. viewing a different diagram of the same phenomenon. The domain-specific knowledge may, therefore, be considered to be an individual's prior or content knowledge. In contrast, domain-general knowledge is described as general visual knowledge that enables an individual to characterise the elements of a diagram perceptually (Lowe, 1994 & 1993). Thus, this domain-general knowledge would refer to a certain degree of visual literacy required by the user to effectively use the diagram as discussed earlier (Section 2.3.1). Although there are quantitative differences in the amount of knowledge novices and experts have, Lowe (1994b & 1993) also cites Chi *et al.* (1988) who state that there exist qualitative differences in the manner in which the knowledge of experts and novices is structured. In this regard, the higher order relationships that experts have in their knowledge structures are depicted in an abstract form by the visiospatial relationships among the elements in a diagram (Lowe, 1994b).

Coupled to content knowledge, is an individual's ability to reason with a diagram, which is an important part of the visualisation process (Anderson, 2003, pers. comm.). We discuss this issue in the following section.

2.3.3.4 Diagrams and Reasoning

Reasoning is closely related to problem solving and creativity. It encompasses all thinking activities that involve making or testing inferences, e.g. inductive reasoning (i.e., concept formation) and deductive reasoning (i.e., logical argument) (Kearsley, 1994- 2003). Stenning and Lemon (2001) have described diagrammatic reasoning as being a combination of the representational and computational issues from psychology and logic, and explain how individuals create, interpret and use systems of diagrammatic representations. More simply, Fisher *et al.* (2000) describe diagrammatic reasoning as the

understanding of concepts and ideas through the use of diagrams. Thus, there is a link between diagrammatic reasoning and visual literacy, whereby we may suggest that in order to reason effectively with a diagram, an individual needs to be visually literate. It has been suggested that visual perception (especially perceptually represented knowledge of experiences) and visually-based reasoning are tightly coupled and it is not only conceptual knowledge (as discussed previously in Section 2.3.3.3) that plays a part in the reasoning process (Chandrasekaran & Narayanan, 1992). This implies that novices may inappropriately use everyday dynamics to reason with diagrams (Lowe, 1999).

In order to perform diagrammatic reasoning effectively, an individual is required to transfer skills from one context to another and to generalise from their experience. The reasoning task, the students' prior knowledge and diagrammatic reasoning system are all factors determining whether a problem will be effectively solved (e.g. Brna *et al.*, 2001). As a result, the numerous strategies employed by experienced learners in a reasoning task, for example, are beyond the conscious control of the learner and therefore the learner may have difficulty remembering the strategies once the task is completed (Lewalter, 2003). Lowe (1994) supports an existence of diagram genres whereby an individual who is familiar with highly specific domain diagrams, will be proficient with different diagrams due to transfer. In order to transfer diagrammatic reasoning skills, Brna *et al.* (2001) believe that the individual needs to be familiar with the current reasoning context, decontextualise what is learnt by, for example, using different tasks at different stages of experience, be familiar with other systems of representation and be able to move between them. There are many studies that report on the differences between experts and novices with respect to reasoning and effective use of diagrams, examples of which are discussed below.

Differences between experts and novices when using diagrams as reasoning tools, is of great relevance to the work reported in this dissertation, and has been noted in this chapter on both the perceptual and reasoning levels. On a perceptual level (discussed in Section 2.2.1), novices have been found to confuse visibility in diagrams with relevance (e.g. Kozma, 2003 & Petre & Green, 1993), and similar inferences have also been found in studies on the effectiveness of animations. For example, Lowe (2003) reports that novices extracted information based on perception and not on thematic relevance. For example, objects that changed position were noted more frequently than less dynamic objects in the

same animation (Lowe, 2003). Novices have also been found to transfer the function of everyday elements, or the shape of a diagram, to the diagram as a whole leading to difficulties in interpretation and confusion about diagram elements e.g. the range of arrow meanings (Pintó & Ametller, 2002). To compensate for this lack of content knowledge, which leads students to classify diagrams on their visiospatial level, text has been suggested as a means for guiding the diagram user (Lowe, 1996). However, information presented by a caption has been found to be neglected, leading to misinterpretations based on students linking the shape of the diagram, to concepts that were inappropriate to the represented situation (Pintó & Ametller, 2002). In contrast, experts almost always used textual information, to guide them through the graphical representation and were thus able to ignore irrelevant information, match patterns and recognise the grouping of elements (Petre & Green, 1993). In addition, strong evidence supports the notion of content knowledge being required for diagram interpretation. For instance, Lowe (1993) has found that experts, in contrast to novices, were familiar with the symbolic representations used in the diagram as well as the method in which these symbols were depicted. Furthermore, some diagrams are important tools for reasoning and mental model construction (Gobert & Clement, 1999 & Lowe, 1991 & 1989), as discussed in Section 2.3.3.2. This was illustrated in a study by Kindfield (1992) who noticed that experts, in contrast to novices, generated and used their fine-tuned diagrams to: support their thought processes while solving the problem, record information in multi-step problem solving, and to check their previous reasoning by the previous 2 steps. Thus, the use of student-generated diagrams is an important data collection tool in conjunction to verbal data collection (as discussed further in Chapter 3, Section 3.3.6). According to this strategy, an individual exposes their mental model of the situation when generating their own diagrams (Kindfield, 1992).

2.4 Where to now?

The aim of the literature review was to highlight the current status of the field of diagram research, in terms of the studies reported on in this dissertation.

Through a review of the literature, I have endeavoured to show that there are no clear definitions of the terms “diagram” (see Section 2.1) and “visualisation” (see Section

2.3.2.1). These 2 constructs are the cornerstones of this dissertation that investigates students' visualisation of biochemical processes, through the use of diagrams. In addition, I highlighted the importance of defining these terms in the context of the subsequent work, as described in Sections 2.1 and 2.3.2. There are also varied opinions by authors on the effectiveness of diagrams in instruction and learning of science (presented in Section 2.2). Thus, I concluded that factors such as the student's degree of visual literacy (see Section 2.3.1), their conceptual or prior knowledge (see Section 2.3.3.3) and what is being evaluated by the researcher (see Section 2.2), all play major roles in making assumptions on the effectiveness of diagrams.

No one disputes the advantages of using diagrams as teaching and learning tools. However, despite some of the cautions against the use of diagrams (see Section 2.2), there is no way of avoiding the use of diagrams that typically adorn all biochemistry textbooks. The development of more dynamic multi-media visualisation tools (see Section 2.3.2.2) is taking place at an alarming rate, despite some of the reported visualisation difficulties and concerns as to the effects of these visualisation tools on student learning (see section 2.3.2.3). This foundation highlights the motivation for the studies reported on in this dissertation, because as I have shown, there is a need to investigate the effects of textbook diagrams on students' understanding and visualisation of biochemical processes. In accordance, Chapters 5- 7 investigate this understanding and visualisation. However, as a prerequisite for comparison, Chapter 4 investigates expert propositional knowledge on linear, cyclic and cascade biochemical processes. The following chapter thoroughly discusses the research process used in these investigations and the rationale for the methods employed.

CHAPTER 3

Methods

3.1 Introduction

Cohen and Manion (1994, pg 38) describe research methods as the range of approaches used in the collection, interpretation, explanation, prediction and inference of data. In this regard, this chapter provides a thorough account of the conceptual (Section 3.2) and methodological (Section 3.3) frameworks employed in the studies reported in this dissertation. Thus, the approach and range of methods that were used in this dissertation, to gather and interpret data on expert (see Chapter 4) and student (see Chapters 5- 7) understanding of various biochemical processes is discussed. In addition, the limitations as well as the strengths of the methods used are considered. Although examples of the different types of written probes are given in this chapter, the specific probes, propositional statements and diagrams used in the different studies can be found under the Methods sections of Chapters 4-7. The rationale for the design of certain probes and methods employed is dealt with explicitly in the Methods sections of the appropriate chapters.

The range of methods used to understand, interpret and explain phenomena in their natural state is a form of inductive inquiry that constitutes qualitative research, where results are attributed to research skill and the perspective of the researcher, thereby possibly leading to differing interpretations by different researchers (Anderson & Arsenault, 1998, pg 119). Thus qualitative research, as prescribed in this dissertation, makes use of the researcher as the principle source of data collection. The method is also concerned with individual's perceptions, whereas quantitative research employs data collection tools (e.g. IQ tests) and is concerned with quantifiable and generalisable findings (Bell, 1999, pg 8 & Anderson & Arsenault, 1998, pg 123). Overall, qualitative research is a form of naturalistic inquiry where data is collected from individuals in their natural setting or context in order to discover the natural flow of cognitive processes (Lincoln & Guba, 1985). It is stated by Lincoln and Guba (1985) to be a legitimate form of inquiry.

However, there are limitations to qualitative research where most of the data is narrative, as with any other type of research (Phelps, 1994). Probably the most commonly viewed limitation of qualitative research is that there will always be more than one valid perspective held by any number of researchers, which is why triangulation is important. However, Anderson and Arsenault (1998, pg 133) state that the qualitative researcher's defence is that they have no investment in the results of the research, which is conducted in a focused and systematic manner. In addition, the apparent lack of internal validity (see later section 3.3.5) is also seen as a possible limitation, although this may be overcome by illustrating how data and analysis were used to generate conclusions (Anderson & Arsenault, 1998, pg 134). This has been explicitly done in the Results and Discussion sections of Chapters 4-7. Furthermore, the results of qualitative research may not always be generalised to other situations and, in this regard, authors such as Anderson and Arsenault (1998, pg 134) and McMillan and Schumacher (1993, pg 373), state that the qualitative researcher should not be concerned with this apparent limitation since this is not a primary objective of this type of research.

Now that the limitations of qualitative type research have been pointed out, the conceptual framework (Section 3.2) and methods (Section 3.3) used in the different investigations making up this dissertation will be discussed. By providing a rationale for each of the methods used, we aim to highlight the effectiveness and strengths of this type of qualitative research, which has an important function in gaining insight into students' (and experts in Chapter 4) understanding and visualization of biochemical processes.

3.2 The Conceptual Framework: Constructivist Theory

Constructivist theory (e.g. von Glasersfeld, 1992) on how people learn is currently probably the dominant theory used by science education researchers, and is the theory on which this study is based. This theory is in contrast to the "transmission model" of learning whereby the learner is seen as a cup into which the instructor is able to pour knowledge. Driver and Bell (1986) state that learning, in terms of constructivism, is a continuous and active process of constructing meanings by the learner, which depends on an individual's prior knowledge and learning environment. Once meanings are constructed

from information and evaluated by a learner, they are either accepted or rejected since learners are responsible for their own learning (Driver & Bell, 1986).

It follows that a person's "cognitive structure" is the manner in which accepted meanings are organised in human memory (Jonassen & Hawk, 1984). Thus, by exposing a person's mental model of a given situation, we are able to identify difficulties should they exist. In terms of investigating student understanding under the guise of constructivist theory, Phelps (1994) views qualitative research as the investigation of the subjective reality of individuals when constructing their own truths. In this regard, qualitative research has been shown to be effective in investigating student understanding, as it allows an in depth investigation into the meanings constructed by individuals. Therefore, since the primary objective of the studies reported in this dissertation was to investigate students' (and experts in Chapter 4) understanding and visualisation of biochemical processes, qualitative research methods were employed as they involve instruments that could best answer the research questions that are posed.

3.3 The Methodological Framework

Since qualitative research often employs inductive analysis, whereby patterns of student conceptions emerge from the data, it is difficult to describe the methods used in this dissertation as a linear progression. This is because student difficulties may emerge at any stage through the use of any number, or one of the methods employed in the investigation. Authors such as Lincoln and Guba (1985, pg 187) and McMillan and Schumacher (1993, pg 374), stress that a definitive design of a qualitative research project is almost impossible as these types of naturalistic inquiries have an emergent design whereby each successive stage of the research plan is dependent on the step or data obtained from the step before. In other words, it is an iterative process.

Figure 3.1 represents the research approach and interpretive framework we employed in the studies reported on in this dissertation. In this regard, corresponding elements to the research process will be discussed in various sections throughout this chapter.

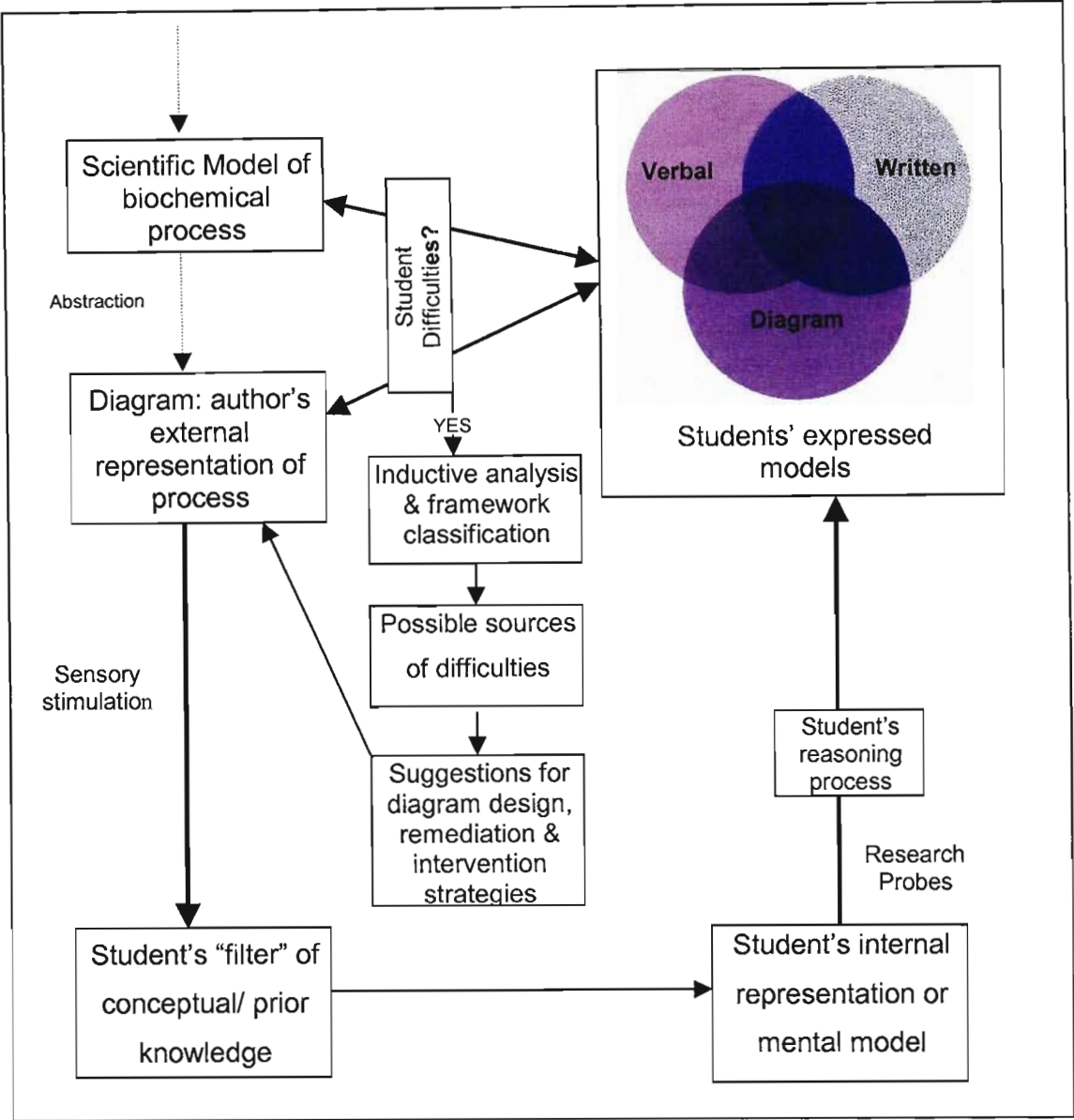


Figure 3.1: Model of the research approach and interpretive framework employed in the studies reported on in this dissertation

The model presented above in Fig. 3.1, presents a general overview of the research process used in the various studies reported in this dissertation to investigate students' visualisation of biochemical processes. The meaning we assign to the broadly used term "visualisation" in the context of the studies reported on in this dissertation was discussed in Chapter 2 (Sections 2.3.2.1 & 2.4). In summary, we interpret the process of visualisation to include all processes involved from reading a diagram to externally expressing an interpretation of it (McCormick *et al.*, 1976). Thus, visualisation involves the development and use of

mental models, which includes its use in inference generation and problem solving (Anderson, 2003, pers. comm.).

With respect to Fig. 3.1, once an author constructs a diagram from his/her understanding of an accepted scientific model of a situation (in our case biochemical process), it is presented to students in instruction, or in our case, to investigate students' visualization of biochemical processes. The diagram is responsible for the sensory stimulation that the student processes, at a number of levels. Thus, in line with constructivist theory, the student passes the extracted information through their "filter" of conceptual knowledge, thereby constructing an internal representation e.g. mental model of the depicted situation (see Chapter 2, Section 2.3.3). Thus, the written or interview research probes that were given to students (and experts) require them to elicit or express (elements of) their mental models. However, Reiss and Tunnicliffe (2001) state that there is often a difference between an individual's expressed and internal models, and therefore, more than one of their expressed models should be investigated. In this regard, Fig. 3.1 summarises, as a Venn diagram, the method employed to gather student data and compare students' responses to written (Section 3.3.3) and interview (Section 3.3.4) probes, as well as student-generated diagrams (Section 3.3.6). Overall, this method allowed us to determine whether there were similarities or differences between any of the expressed models. By comparing students' responses or expressed models to propositional statements (expressions of the diagram and currently accepted scientific model), any difficulties that were identified were analysed inductively (Section 3.3.7) and classified on the framework (Grayson *et al.*, 2001) (Section 3.3.2). Subsequently, by identifying possible sources and the nature of the difficulties using the model of Schönborn *et al.* (2003 & 2002) (Section 3.3.8), we are able to tentatively suggest possible remediation strategies and suggestions for diagram design.

We now consider aspects of the research process in more detail.

3.3.1 Sample Size

McMillan and Schumacher (1993, pg 382) suggest that the purpose of the study, the data collection tool, and the research question or problem, should be taken into account when deciding on a qualitative research sample size. Such sample sizes are generally smaller

than the samples used in quantitative research projects, where the objective is to generalise results to the greater population (McMillan & Schumacher, 1993, pg 382). In our studies we used the entire class of biochemistry students as a sample to answer the written probes. Depending on the number of students attending classes, this was approximately 95 students in the studies reported in Chapters 5 and 6, and 27 and 30 students for those reported in Chapter 7. The large numbers of students, and the different types of written probes employed in the study, enabled the identification of a wide range of student difficulties and thus acted as a good indication of the incidence of the difficulties. In interviews, however, we asked students to volunteer and interviewed 10 students for the studies reported in Chapter 7 and 15 students for those reported in Chapters 5 and 6. The nature of the interview technique employed (Section 3.3.4), did not require us to have large sample sizes as we aimed to gain insight into the nature and possible sources of the different difficulties, rather than their incidence.

In order for our studies to be ethically sound, students were informed about the nature and purpose of our investigations and told that their identity would remain confidential. Section 3.3.4.2, elaborates on how ethical considerations were maintained during data collection.

In the study reported in Chapter 7, we sought opinions through e-mail of 5 experts, of whom 2 responded. With respect to the study in Chapter 4, 23 experts were e-mailed a questionnaire to gather expert responses on various biochemical processes, and 13 experts responded. Generally, Lowe (e.g. 1996 & 1994) classifies experts as having between 3-30 years experience and novices (or students) as having no experience. This is consistent with the studies reported in this dissertation, where all experts held at least senior lecture positions at their respective universities and all had doctoral qualifications.

3.3.2 The 4-level Framework Used for Identifying and Classifying Student Difficulties

The 4-level framework (Fig. 3.2) of Grayson *et al.* (2001), was used as the primary method in this dissertation for identifying and classifying student difficulties inductively. It supports a naturalistic qualitative form of inquiry (Lincoln & Guba, 1985).

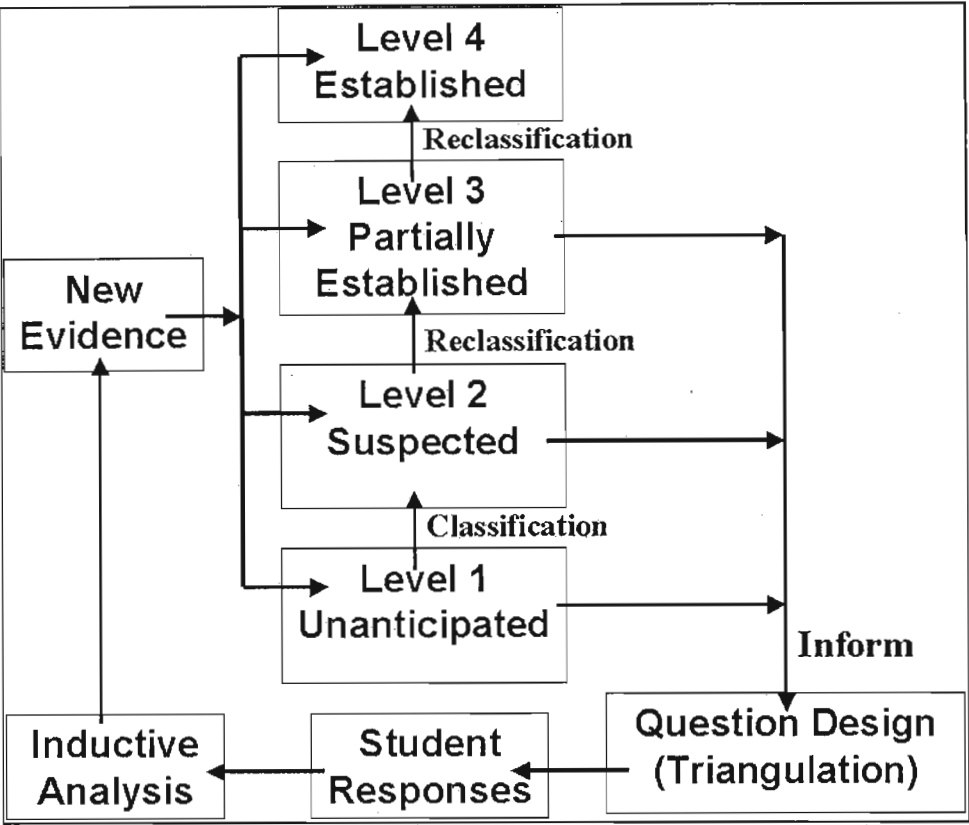


Figure 3.2: Diagram representing the framework of Grayson *et al.* (2001) used to identify and classify student difficulties (reprinted with permission of Grayson *et al.*, 2001).

The above diagram (Fig. 3.2) illustrates the cyclical and hierarchical nature of the framework. In this regard, difficulties may be promoted up the hierarchy from levels 1 to 4, based on the insight into the nature of the difficulty. The process of gaining greater insight into the nature of each difficulty is cyclical in that knowledge of a difficulty informs probe design, and inductive analysis of student responses, informs the classification of difficulties on the hierarchy. This cyclical process continues until the difficulty has been classified at level 4. Level 1 of the framework describes difficulties that are unanticipated and arise unexpectedly out of the data analysis. After their emergence, level 1 difficulties are then moved to level 2 as suspected, where the nature of the difficulty still has to be researched. Level 2 difficulties may also be suspected on the basis of researcher, teacher or learner experience in the respective domain, or from literature reports on problems or difficulties students have with an aspect of that domain. Once the level 2 difficulties have been systematically investigated, they are classified as partially established or level 3 difficulties where a description of the nature of the difficulty

has been formulated. Thus, the classification of the difficulty at levels 1 and 2 is subjective but becomes more objective at levels 3 and 4 as the description of the difficulty becomes more stable (Grayson *et al.*, 2001). The level 4, or established difficulties, are those that have been systematically investigated in a number of contexts e.g. different student populations and educational environments. Usually, level 4 difficulties have been published in the literature and are well established.

Identifying and classifying difficulties on the framework (Grayson *et al.*, 2001) requires the emergent design of various types of written and interview questions (Sections 3.3.3 and 3.3.4.3, respectively). Following this, the inductive analysis of the student responses (Section 3.3.7) allows difficulties to emerge from the data. The identification of difficulties allows for the calculation of the incidence of difficulties, thereby introducing a quantitative dimension to the qualitative research design.

Since "...the wider the range of measures used to probe understanding, the better the assessment" (White & Gunstone, 1992, pg 177), various written probes (Section 3.3.3), interviews (Section 3.3.4) and student-generated diagrams (Section 3.3.6) were used as tools to gather data on student understanding. Cohen and Manion (1994, pg 233-234) also support the use of many different methods to ensure confidence in the results obtained. The use of multiple-methods also supports the validity (see Section 3.3.5) of the results. On this basis we, therefore, employed methodological triangulation which "uses either (a) the same method on different occasions, or (b) different methods on the same object of study" (Cohen & Manion, 1994, pg 236) and compared our results obtained from these methods.

3.3.3 Written Probes

A "probe" is the name given to a question in this dissertation that was specifically designed to investigate or probe an individual's understanding of a particular concept. To ensure that we were indeed probing for student understanding, we made every effort to eliminate the need for any memory recall by supplying them with the necessary theoretical details of biochemical processes in the form of relevant diagrams and captions. In cases where the students could not always extract the required information in order to answer a probe directly from a diagram, we were testing a diagrammatic reasoning or visualisation ability

where students would reason using the diagram and possibly their content knowledge in order to interpret the event or concept that was being probed on (Anderson, 2003, pers. comm.). Examples of this type of probing for visualisation, in which students were required to access their conceptual knowledge in addition to interpreting the diagram, are discussed in detail in Chapter 7, which focuses on the complement pathways. Cohen and Manion (1994, pg 283) state that questionnaires (and probes in this dissertation) are economical in terms of money and time, compared to the process of interviewing and their transcription. In this regard, we paid students for their time in interviews, and e-mailed the experts discussed in Chapter 6, which saved mailing and paper costs. Cohen and Manion (1994, pg 283) and Pribyl (1994) also state that the disadvantages of the questionnaire (especially when mailed or e-mailed) over the interview are that not all questionnaires are returned. In our study, 13 out of approximately 23 experts in our sample mentioned in Chapter 4 replied, but since students were required to answer probes in their tutorials all probes were returned. Furthermore, the researcher is not easily able to clarify questions the respondent may have with respect to the nature of the question. With respect to this last point, the data gatherer was always present when the students answered the probes in their tutorials, while some experts communicated any queries they had about the probes (see Chapter 4, Section 4.2.3).

We took various steps to try and optimize the quality of the written responses and, therefore, of our data. Firstly, students were given no time limit within which to complete the written probes. This relieved any pressure that may have been placed on them by time restrictions, a factor which could have affected the quality of their responses. Secondly, student responses, in the case of the studies reported in Chapters 5 and 6, were marked and contributed 5% of their class mark for the particular semester. This proved to be an effective incentive to students to answer all of the probes on the “tutorial sheet”. Thirdly, all students, including those reported on in Chapter 7, were told that the probes would be used to assess their understanding and information inferred would be fed back into improving their own learning and those of future students. Fourthly, we limited the number of probes given to the students in each sample to cover only one page, thus eliminating any fatigue associated with the answering of too many extended questions.

The written probes were typed and enough space was allocated on the page for students to fill in their answers. This aided analysis as the probe always accompanied the answers to

each probe. Wherever a diagram was required for students to answer the probes, this was given to each student with the probe sheet and the following instruction was found at the top of the probe sheet: “Use the diagram provided to answer the question(s) and support your answer(s) with an explanation”. Once the student responses had been marked and/ or analysed they were returned to the students, but only once we were sure it would not affect any further data collection. In some cases, this was only after interviews had been conducted. For our study to be ethically sound, the lecturers of the various courses were informed of any difficulties we had identified and students were given “model” answers to the various probes (Chapters 5 & 6). A tutorial was also scheduled for other students (those in the sample in Chapter 7) in order to point out to students any areas of confusion that we had found before they wrote their final examinations.

Three types of written probes were given to the students to investigate their understanding. These can be found, where applicable, in the respective Methods sections of each chapter. Initially, free-response type probes were given to the students. These probes are somewhat open-ended as they allow the individual to express their understanding freely without restraint. An example of a free-response type probe that was given to students in conjunction with a diagram is illustrated below in Fig. 3.3.

Describe everything you think this diagram is meant to represent or show.

Figure 3.3: Example of a free-response type probe given to the students

While the probe in Fig. 3.3 above allowed the students to freely express their understanding of the illustrated content depicted in the diagram, it also drew their attention to the diagram as a whole. As this type of probe is very general, it is not uncommon that suspected difficulties are not yet revealed and that the number of students who show a particular difficulty is less than the actual number of students who hold the difficulty. Youngman (1986), as cited by Bell (1999, pg 119), states that the extended nature of answers to free-response type probes, as compared to those of more focused probes, makes them more difficult to analyse. We did not find that the free-response type probes presented difficulty, not only because we restricted the space for students to answer (see

back), but also because our inductive analysis approach allowed us to rapidly identify patterns of difficulties and efficiently place them into broad categories. Knowledge of these broad categories informed the design of more structured probes that specifically focused on the nature of each difficulty.

Semi-focused type probes followed the free-response probes in order to delve deeper into difficulties that students manifested in the free-response type probes. These were also used to investigate suspected difficulties (Grayson *et al.*, 2001) that did not reveal themselves in the free-response type probes. An example of a semi-focused type probe (found in Chapter 7 on the complement pathways), given to the students in conjunction with a diagram, is shown in Fig. 3.4 below.

Use the diagram provided to answer the following question(s) and support your answer(s) with an explanation.

Explain how C3b is formed.

Figure 3.4: Example of a semi-focused type probe given to the students

The semi-focused type probes attempt to focus on suspected difficulties without leading the students into a particular answer.

Two-tier multiple-choice type probes (after Treagust, 1988) followed the semi-focused probes. This is because interviews conducted by Fisher (1985) showed that conventional multiple-choice type probes, that require students to select an answer without providing an explanation, have flaws as they may overestimate alternative conception frequencies. This is because a student may guess the answer or choose an option by mistake, although they have a sound understanding of the concept. To counteract this flaw, Treagust's (1988) two-tier multiple-choice type probes consist of a question followed by two or 3 answers and, once students have selected their answer, they are further required to select a provided reason to their answer in the first part of the question. The reasons that form the second-tier of the multiple-choice probe consist of the scientifically acceptable answer and any number of previously identified alternative conceptions, termed "distractors" (Treagust,

1988). The two-tier multiple-choice probes used in our studies, and reported in this dissertation, are based on the principle proposed by Treagust (1988) but require students to select an answer to a probe and then provide their own reason to support their answer. Provision of reasons by students was considered crucial for reinforcing the existence of a particular difficulty. Therefore, in cases where this part of the probe was left blank the data was considered flawed and thus disregarded. This was also done to eliminate the influence of any guessing as in normal multiple-choice items. The answer choices offered to the students consisted of the correct answer(s) and a variety of distracters, some of which were difficulties that had already been identified from student responses to the various other written probes, and in some cases may have been suspected difficulties that had not revealed themselves in any of the other written probes. An example of a two-tier multiple-choice type probe, given to students in conjunction with a diagram depicting glycolysis (see Chapter 6), is given in Fig. 3.5 below.

<p>Use the diagram provided to answer the following question(s) and support your answer(s) with an explanation.</p> <p>Choose the statement(s) that describe(s) what happens in reaction (5):</p> <p>(a). Glyceraldehyde 3-phosphate combines with Dihydroxyacetone phosphate to form Glyceraldehyde 3-phosphate (2)</p> <p>(b). Dihydroxyacetone phosphate is converted to Glyceraldehyde 3-phosphate</p> <p>(c). Dihydroxyacetone phosphate forms 2 Glyceraldehyde 3-phosphate</p> <p>a b c (circle your selection(s))</p> <p>Explanation of your choice(s).....</p>

Figure 3.5: Example of a two-tier multiple-choice type probe (Treagust, 1988) given to the students

In the above example of a two-tier multiple-choice type probe (Fig. 3.5), options (a) and (c) represent student difficulties or distracters that had already been identified from students' responses to the free response and semi-focused type probes while option (b) is the scientifically acceptable answer to the question.

3.3.3.1 Incidence

An incidence of a particular difficulty is calculated by dividing the number of students who showed a particular difficulty by the total number of students who answered the probe. The ratio is then expressed as a percentage. Incidence calculated for free-response type questions represented the *minimum* percentage of students with that difficulty. This is because the nature of the probe is such that students do not necessarily reveal certain difficulties that they have. By contrast, incidence calculated from student responses to the two-tier multiple-choice type probes, were better indicators of the extent of the problem, although not concrete indicators because of the subjective nature of the responses, which might change from one time to another, or from one probe to another. However, this was not considered a problem as our prime aim was to get an approximate idea of which difficulties warranted further investigation.

The question of whether these written (and interview probes as discussed in Section 3.3.4) probes are reliable and valid is important as, if the question is misleading or ambiguous, especially with respect to leading students into stating a difficulty, then the incidence and the level of the framework at which we classify that particular difficulty will be invalid. The reliability and validity of the written and interview probes presented in this dissertation are discussed in Section 3.3.5.

3.3.3.2 Propositional Statements

In order to determine whether particular responses to a written probe was a difficulty, a mere difference in opinion between researcher and student, or a variation of what we considered to be correct, we formulated a set of scientifically acceptable answers or propositional statements to each probe. The propositional statements were comprised of our knowledge of a particular concept, and various textbook explanations. Furthermore, an expert in the biochemistry department working in that particular domain validated them. Since students may only state a few of the propositional statements, especially in response to the free-response type probes, we compiled a comprehensive list of answers that we felt would encompass all the different views taken by students. The propositional statements applicable to each of the probes can be found in conjunction with the appropriate probes under the Sections “Design of written and interview questions” situated in the respective Methods sections of Chapters 5- 7.

3.3.4 Interviews

Interviews are conversations that expose an individual's knowledge about a concept or an event, and include opinions, images and motor skills if relevant (White & Gunstone, 1992, pg 82). Since interviews are more adaptable than written probes (as suggested by Bell, 1999, pg 135), they were used to delve deeper rather than more broadly into difficulties (Anderson & Arsenault, 1998, pg 169). Thus the objective was to investigate the nature and possible sources of the difficulties that had already been identified and classified on the framework of Grayson *et al.* (2001), and also to reveal any difficulties that had not previously been identified in response to any of the written probes. The primary use of interviews is for the collection of this type of in-depth qualitative data (Verma & Mallick, 1999, pg 122) whereas written probes, such as the two-tier multiple-choice type probes (Treagust, 1988), may also be used to collect quantitative data. This section deals with the advantages and disadvantages of interviewing as a data collection tool and the process and design of the interview protocol in the current work. Extracts of actual interview transcripts are provided in the Results and Discussion sections of Chapters 5- 7.

Anderson and Arsenault (1998, pg 190) and Cohen and Manion (1994, pg 283) support the use of interviews as a research technique, as the interviewer is able to clarify questions and solicit more complete responses. This is because the interviewer can question the respondent until he/she is satisfied with the depth of the response, and monitor the respondent's non-verbal cues such as facial expressions that are also indicative of the respondent's confidence during his/her answer. Since the reliability and validity of the data collected during an interview is dependent on the interviewer alone, this may be seen as a disadvantage of this technique (Anderson & Arsenault, 1998, pg 190). To address this issue we got other members of the research group to read verbatim interview transcripts to check that there was neither ambiguity nor leading questions asked. Furthermore, we videotaped interviews (in 2 of our 3 studies) to monitor where students were pointing on diagrams, and to study the construction of their own diagrams. We also avoided interruptions by making use of an interview room, and allowing students to choose their own interview times.

Cohen and Manion (1994, pg 282) and Bell (1999, pg 135) suggest that the characteristics of the interviewer should be matched to those of the respondents to minimise any bias that

may occur in their interaction. This was done in the present study, in which both the interviewer and the respondents were registered students. In terms of the analysis of the interview transcripts, Cohen and Manion (1994, pg 282) state that some disadvantages of the interview technique may be that students misunderstand what is being asked, although I found that students freely asked for clarification on a particular question when they were unsure. Furthermore, that the interviewer may misinterpret what the respondent said, we overcame by allowing other members of the group to read the interview transcripts along with the analysis by the interviewer, to check the validity of the analysis. In addition, a disadvantage of the interview technique, compared to written probes, is that the process of interviewing and transcribing the interview, and analysing the transcripts, is far more time consuming than that required for written probes. However, we considered these minor factors when considering the quality and depth of data that can be collected from interviews. Interviews were also more costly than written probes as students were paid for their time and the video-recorder had to be hired.

In relation to the above, there are many factors to be taken into account during the planning and conducting of interviews. These will now be discussed.

3.3.4.1 Interview Data Collection

Students were asked to volunteer for interviews, once all the written probes had been analysed. They were told that the interviews would remain strictly confidential and would not count towards their course assessment. Students were paid R20-00 per interview. Each interview lasted 30- 45 minutes, and I was flexible in allowing students to schedule an interview in between their lectures or in the afternoons when they had free time, so as not to interfere with their lectures.

The interviews were recorded with a Dictaphone that had already been set up in the interview room, where only the interviewer and student would be present. A video-recorder was available for use in the interviews conducted with the students in the samples mentioned in Chapters 5 and 6, but not with the sample in Chapter 7. The video-recorder was set up to focus on a preset square which had been marked on the table, where the diagram that the student was being questioned on was in focus, or where a sheet of paper could be placed in cases where the students were required to draw their a diagram. The advantage of video-, rather than audio- taping an interview is that the former takes some of

the pressure off the interviewer in that they do not have to continuously take notes about where students are pointing or the order in which they draw diagram elements etc. Instead, the interviewer is able to stay focused on the students' responses and a desired line of questioning.

Each interview was transcribed verbatim and inductively analysed as described in Section 3.3.7. Lincoln and Guba (1985, pg 272) view the verbatim transcription of interviews as a disadvantage because it is so time consuming and there is too much time between gathering the data and being able to work with it. However, the verbatim transcription of interviews served as a valuable source of data for us and I transcribed the interviews as soon as possible while I still remembered each interview. Certain conventions were adopted in the interview transcripts such as the letter "I" to designate the interviewer and "S" to designate the student. In addition, square brackets were used to indicate any information that was unspoken but was required in order to analyse the transcripts. The word "unintelligible" was used if we were unable to make sense of what the student said in the interview during listening to the audiotape. The following nonsense interview extract illustrates some of the conventions used in interview transcripts seen in various Results and Discussion sections of Chapters 5- 7:

I: Do you have a dog?

S: [nods]

I: what is its name?

S: [giggles] my dog [is] called Peanut

I: take a look at all these photos of dogs [shows student photograph sheet of dogs seen in Fig. X]. What type of dog is Peanut?

S: ...[student studies photograph sheet] that one [points to greyhound and utters unintelligible word]

In the above interview extract, the student's (S) affirmative response to the question of whether s/he had a dog was indicated by the student's nod, this gesture would have been noted by the interviewer (I) during the interview. The word "is" was also added in square brackets into the interview transcript when the student named his/her dog in order to make sense of the sentence. The photographs that were used in the interview are also referred to in square brackets in the interview transcript so that the reader may consult the surrounding

text to study the diagram in question. The three dots (...) indicate a pause by the student and the reason for the pause is indicated in the square brackets that follow.

3.3.4.2 Relaxing the Interviewee and Establishing an Ethical Rapport

Relaxing the student to be interviewed and establishing a rapport with them is probably one of the most important prerequisites (White & Gunstone, 1992, pg 68), apart from the interviewer characteristics and his/her preparation, to gathering data that is both reliable and valid. Various authors (e.g. Bell, 1999, pg 139 & Cohen & Manion, 1994, pg 283) have stated that the respondent or interviewee often answers questions according to what they think the interviewer wants to hear. Since both the interviewer and respondent were students, the interviews were kept confidential, and did not count towards any of their class assessments, this issue was not perceived to be a disadvantage of the interviews reported in this dissertation. When students arrived for the interviews they were assured of these latter facts and that the purpose of the interview was to gain greater insight into areas of possible difficulties so that more effective teaching strategies and approaches could be formulated (an approach also suggested by White & Gunstone, 1992, pg 68). Casual chat was engaged with the student about varsity life in general in order to create a friendly environment (Lincoln & Guba, 1985, pg 270). Schollum (1983) also relaxed children in his study with questions about their schooling. In addition, McMillan and Schumacher (1993, pg 252) suggest that the interviewer dress in a manner so that the respondent does not assume the interviewer is representative of a particular viewpoint. In this regard, we found that dressing similarly to the students was effective, particularly as both the interviewee and interviewer were students.

Many students felt insecure about the presence of the video-recorder, but this was overcome by allowing them to look through the camera and see that it was only focused on the designated square and not on their faces. The purpose of the Dictaphone was also explained to students in terms of allowing us to transcribe the interview session for analysis. White and Gunstone (1992, pg 86) suggest that the recorder should be turned on once a student's permission to tape the interview has been obtained, rather than just before the probing. Students were briefed by telling them that there were no right or wrong answers to the interview questions and that we were only interested in investigating their understanding of certain concepts. Bowen (1994) also used this type of briefing when interviewing students on chemistry concepts, and followed by asking them whether they

had any questions about the interview. Students were encouraged to be as creative as they wished and to ask for clarification should they not understand what was being asked of them at any point.

3.3.4.3 The “Structure” of the Interview Protocol

Bowen (1994) summarises an interview as greeting the interviewee, providing them with practice questions (which may take the form of general conversation), probing the student, thanking them for their participation and finally asking them for a follow-up interview if required. Unstructured type interviews allow the respondent to freely respond to questioning and the interviewer maintains a broad set of objectives that is in contrast to structured interviews, where the interviewer is required to stick to a prepared list of questions (Verma & Mallick, 1999, pg 123). The interviews that we conducted with students lie on the continuum of structured to unstructured interviews as they are of the semi-structured type (Verma & Mallick, 1999, pg 123), whereby we start off with free-response type probes (such as that illustrated in Fig. 3.3) and progress to more focused probes once a student had revealed a difficulty (see Fig. 3.6).

The following figure (Fig. 3.6) shows a model we devised for the general procedure for an unstructured interview, used in this dissertation, where the interviewer picks up on a difficulty stated by a student in a free-response type question and probes deeper with the use of more structured probes. White and Gunstone (1992) state that it is easy to move back and forth between free-response and more structured probes, which has been indicated on the diagram by the double-headed arrows (e.g. \leftrightarrow). In this regard, the interviewer may in some cases move from a free-response type question down to a structured question, without going back and forth between these types of questions, to obtain a concrete description of a difficulty, and in other cases several sets of semi-focused probes may be required (see Fig. 3.6).

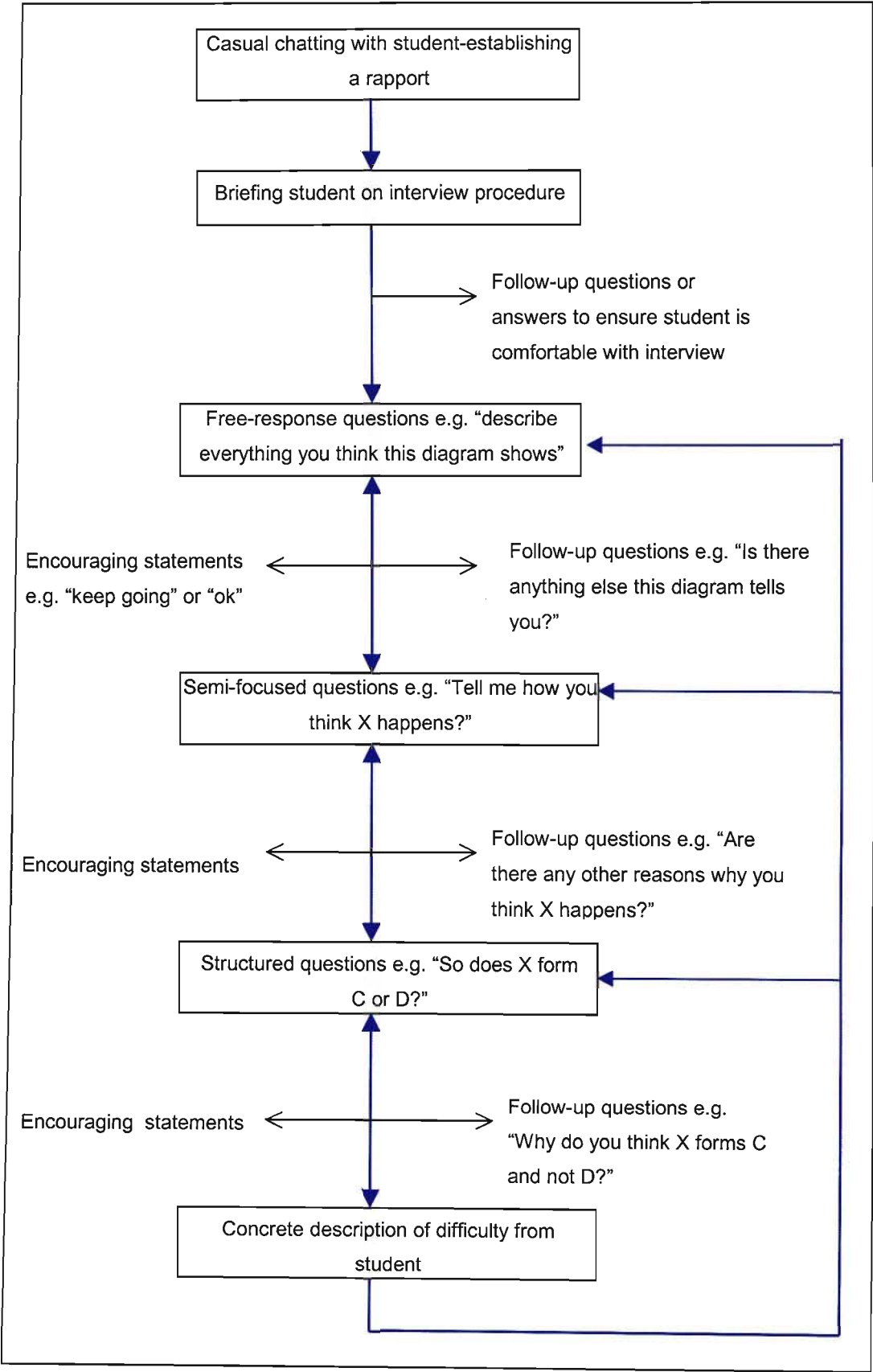


Figure 3.6: Model of an unstructured interview protocol used in the current studies

In terms of Fig. 3.6, free-response type questions enable the interviewer to assess the extent of the respondent's knowledge, to help build rapport, and to make decisions whether to then use structured probes to delve deeper into any suspected alternative conceptions (Cohen & Manion, 1994, pg 277). The use of both free-response and more structured probes in an interview increases objectivity and uniformity and also allows for clarification and in-depth probing (McMillan & Schumacher, 1993, pg 252). An example of a semi-focused type probe would be where a student said cleavage was occurring in response to the probe in Fig. 3.4, the interviewer could ask: "why do you think cleavage is occurring?" or "is there something on the diagram that tells you cleavage has occurred?" Follow up questions, as suggested by Gunstone and White (1992, pg 66) for example, "how do you think that happens?" or "why did you choose that one?" were also extensively used once a student had revealed a difficulty. Bowen (1994) also suggests the use of follow-up type questions such as "tell me what you are thinking?" when interviewees are silent for a period of time to encourage them to reveal their thought processes.

Once students have provided a concrete description of a particular difficulty (see Fig. 3.6 above), the interviewer is able to move back to more free-response or structured probes to pick up on other difficulties that students had mentioned, or to investigate whether there are any new ones. Thus, the interview process may be of a cyclical nature but can also go back and forth between the different types of questions. Therefore, the manner in which the interviewer asks the student questions can greatly influence the responses given by the student, which is why it is important to remain neutral and keep the natural flow of the interview going.

3.3.4.4 Being Neutral and Maintaining the Flow in an Interview

It is essential that an interviewer remains neutral during the interview so as not to ask leading questions that may bias the data (McMillan & Schumacher, 1993, pg 252). This is because the student responds in a manner that the interviewer wants them to. Apart from being careful not to ask leading questions, the interviewer has to keep an even tone and not show emotion that may indicate a student's response is either correct or incorrect. In this regard, McMillan and Schumacher (1993, pg 254) suggest the interviewer practice asking the questions aloud to maintain an even tone and come across in a natural manner. When the interviewer is encouraging, neutral and repeats a statement a student said, the student might often elaborate on their point (White & Gunstone, 1992, pg 86). In cases where

students have exposed a difficulty and then later corrected themselves, it is important to keep questioning the student until s/he is happy with his/her answer and will probably not change his/her mind again. The respondent should not be asked too many questions at once as they will give answers that are less detailed and their reasoning for responding in a certain manner may not be exposed. Instructions such as “take your time and study this diagram...” were used to allow students as much time as they needed to study diagrams and then answer their questions without rushing into an answer. White and Gunstone (1992, pg 87) caution that the interviewer should be aware of signals such as moving around in the chair that indicate the respondent is getting tired. In such cases, the interviewer should acknowledge this and tell the interviewee how many questions are left which may spark up interest in the respondent again.

3.3.5 Reliability and Validity of Written and Interview Questions and Data

The importance of reliability and validity in any research project has been highlighted by Verma and Mallick (1999, pg 3) who have stated that a characteristic of research is the solution of valuable and fundamental questions through the use of techniques that are reliable and valid. A valid research study is one where research frameworks that are understood by other researchers are used and where the research is adequately described so that other researchers may use it to extend the results to other studies (Phelps, 1994 & McMillan & Schumacher, 1993, pg 394). In this light, we used the established framework of Grayson *et al.* (2001), as described earlier, as a method that is available to other researchers. The rationale for designing the probes, analysing the data and the design of subsequent probes (naturalistic inquiry) has been described in depth in Chapters 4- 7. In terms of the reliability of qualitative research, Phelps (1994) argues that the subjective nature of humans will ensure that, in spite of the reliability of the methods used, no study will be replicated exactly. In order to collect data that is both reliable and valid, the probes and interview questions given to the participants in the study should be critically assessed. As we highlighted in Chapter 2, there is a lack of clarity among science education researchers who use the terms “diagram” and “visualization”; Hammersley (1987) states that accepting reported data as reliable and valid may be erroneous, mainly because of the diversity of ideas of different authors about the meaning of these terms. Hammersley (1987), therefore, defines validity and reliability as the goals of the research process. We

will therefore describe what we mean by reliability and validity and the measures we took to ensure that our probes and data are both reliable and valid.

A reliable probe is one that will produce similar results on different occasions under constant conditions i.e. there is consistency (e.g. Bell, 1999, pg 103 & Verma & Mallick, 1999, pg 132-133). A valid probe is one that tests what is supposed to be tested (e.g. Bell, 1999, pg 104, Verma & Mallick, 1999, pg 132-133 & White and Gunstone, 1992, pg 177). Therefore an unreliable probe will not be valid, but a reliable probe may be invalid (Bell, 1999, pg 104 & White & Gunstone, 1992, pg 182). Internal validity is the extent of truthfulness of the interpretations, and is thus required in order to be confident that the results of the study are true to the sample from which data was collected (Anderson & Arsenault, 1998, pg, 13). External validity on the other hand is the extent to which the results obtained can be generalised to other samples (Anderson & Arsenault, 1998, pg 13).

Once each probe was designed, we each read the probe in order to ensure that it was neither ambiguous nor leading, and that the researchers involved in the study agreed on the purpose of the probe. Unfortunately, in this dissertation it was logistically impossible to pilot our probes since we only had limited tutorial sessions with the students in which to collect data due to their lecture schedules and the work that had to be covered in each course. However, colleague reviewing of probes as suggested by Bell (1999, pg 104), ensured that a degree of validity and reliability was attained. In addition, to further ensure that our probes were valid and fair, it was essential that some students in the sample were able to answer the probes in a scientifically acceptable manner. In this respect, we also present students' correct answers to the probes in the appropriate chapters. Many authors (Anderson & Arsenault, 1998, pg 12, Phelps, 1994 & White & Gunstone, 1992, pg 182) state that reliability is ensured when more than one person, or qualitative researcher, reads the student responses and they arrive at the same assessment or conclusions. This form of reliability is termed inter-rater agreement (McMillan & Schumacher, 1993, pg 251). This formed part of our analysis process as once difficulties had been sorted into classes and subclasses, according to the student responses to various written and interview probes, the researchers read the descriptions of the classes of difficulties along with the student responses to the probes and classified and reclassified the student difficulties until there was agreement between the researchers. In terms of interviews, colleague reviewing of

probes and data was a way of ensuring that the interviewer had the required skill to conduct the interviews and, in doing so, obtain data that is both reliable and valid.

In terms of validity, Anderson and Arsenault (1998, pg 165) state that there is no guarantee that the respondents are being truthful, or that they understand the question asked in the questionnaire. As previously mentioned, the researcher was always present to answer any queries students had about the nature of the probes while experts (in Chapter 4) were told to feel free to e-mail us if they had problems with understanding what was being asked of them. In an interview situation, Anderson and Arsenault (1998, pg 165) say that the mood and skill of the interviewer may affect the reliability of the interview data and that multiple sessions by the interviewer can increase reliability. It has also been said (Anderson & Arsenault, 1998, pg 165) that people who provide socially acceptable responses that do not reflect their own views, which applies specifically to personal interviews, may also affect validity in an interview. Since our interviews were not of a personal nature, and were not used to assess or pass judgment on the student being interviewed, we felt that the degree of validity would not be affected, as there was little chance students were responding to questions by saying what they thought was required. Verbatim interview extracts and responses to written probes, which have been provided in the Results and Discussion sections of Chapters 4- 7, illustrate the meanings students and experts have and thus form reliable data, since our interviews were recorded mechanically (McMillan & Schumacher, 1993, pg 389 & Phelps, 1994). Cohen and Manion (1994, pg 281) have suggested that the validity of interview data may be assumed if the data obtained corresponds to that obtained by another method that has also been shown to be valid. In cases where a student showed a difficulty in response to a written probe and this difficulty was found to be corrected by that particular student in an interview session, may not necessarily indicate that the interview or written probe was invalid. The possible reasons for cases like this, when found, have been discussed in the Results and Discussion section of the applicable chapter.

3.3.6 Student-Generated Diagrams

In both the interviews, and in response to some of the written probes, students were required to draw diagrams. Examples of these are shown and discussed in the Results and Discussion sections of Chapters 5- 7.

Kindfield (1992, 1993/1994) has stated that a student's interpretation, generation and use of diagrams forms part of their conceptual knowledge and, therefore, their diagram skills are vital components of what is learnt about biological/ biochemical processes. Diagrams have been described as important tools for reasoning and mental model construction (e.g. Gobert and Clement, 1999 & Lowe, 1991 & 1989). Thus, by analysing student generated diagrams we are able to analyse a student's mental model of the situation (Kindfield, 1992). In contrast, other authors such as Reiss and Tunnicliffe (2001) believe that an individual's mental model and expressed model (what is externalised) are different and that by eliciting more than one of their expressed models, e.g. by asking a student to draw a diagram and then questioning them on it in an interview, we may move closer to understanding their mental model of the situation (see Fig. 3.1). However, distortions may occur when a student tries to verbalise their visual thoughts into the written or spoken word. In this regard, Lowe (1993b) believes that these distortions may be overcome by allowing the student to generate their own diagrams or manipulate the given diagram. There is a disadvantage related to using student generated diagrams as a data source as the question arises as to whether the drawing task tests mental model construction or cognitive processing of a student, or whether it will test drawing skill (Lowe, 1993). In this regard, van Sommers (1984) has shown that physical and cognitive factors influence drawing results by students. By using a combination of verbal data and student-generated diagrams, we believe this disadvantage can be overcome.

In interviews, students were given a plain sheet of white A4-sized paper and a number of coloured pens to use as they wished when they were required to construct a diagram. The instructions given to students before they were required to draw the diagram were kept free-response in nature, to allow the students to draw the answer to the question as freely and creatively as they wished. For example, we might say to a student, "Draw what you think is happening in this reaction". As described in Chapter 5, where students were required to draw what they thought certain processes would look like if they could see the molecules in a cell, the context was set for the students by first asking them to describe what they thought the inside of the cell looked like, then by asking them to draw the outline of the cell as if they were looking at it through a microscope, and finally by asking them to draw what they thought a particular process would look like if they could see the molecules through a very powerful microscope. Thus, by setting the context in this manner we could be more confident that the students were drawing what they thought

processes might look like in a cell thereby reducing any bias in the data. By using verbal data and student generated diagrams we were, therefore, satisfying those authors who believe diagrams represent an individual's mental model (e.g. Kindfield, 1992). Also, we were supporting those accounts which suggest that more than one expressed model should be investigated in order to move closer to an individual's mental model (e.g. Reiss & Tunnicliffe, 2001).

3.3.7 Inductive Analysis of Student and Expert Responses

Naturalistic inquiry is the process of qualitative research, used in the studies reported in this dissertation, where the natural flow of processes of interpretation are discovered through non-interfering qualitative (and sometimes also quantitative) data collection techniques (McMillan & Schumacher, 1993, pg 372 & Lincoln & Guba, 1985, pg 187-188). In this regard, set within the framework of Grayson *et al.* (2001) that was used to identify and classify student difficulties in this dissertation (see section 3.3.2), is the process of inductive analysis that was used to analyse the data. Inductive analysis is described by Lincoln and Guba (1985, pg 203) as "a process aimed at uncovering embedded information and making it explicit". McMillan and Schumacher (1993) describe qualitative data analysis as inductive whereby, through the synthesising of the data by the researcher, categories and patterns of student thinking and difficulties emerge rather than from being predetermined. Complementing the framework of Grayson *et al.* (2001), Arsenault and Anderson (1998, pg 131) and Phelps (1994) describe the qualitative research process as the systematic process whereby data is read, compared and then synthesised into manageable and meaningful chunks so that a description can be written that separates chunks from each other. These categories are not predetermined but emerge from the data as suggested by McMillan and Schumacher (1993), and are reviewed until general patterns are found (Phelps, 1994). Thus, the end result of inductive qualitative analysis is a comprehensive review of the data within limitations of the research process, that is formulated by the researcher through the simultaneous processing of data collection and analysis, that forms a cyclical process (McMillan & Schumacher, 1993). As we collected data from student responses to written probes, broad categories of responses were grouped and compared to each other and thereafter we further investigated these categories with more focused probes while returning to our original categories. In this manner, we formulated descriptions of the difficulties or opinions that became more stable as the

difficulties moved from level 1 to 3 of the framework (Grayson *et al.*, 2001). This method illustrates the cyclical and hierarchical nature of the framework as well as the inductive analysis process characterised by the methods.

3.3.8 Considering the Possible Sources of Student Difficulties

Once student difficulties (in Chapters 6 & 7) had been identified and classified on the framework of Grayson *et al.* (2001), through the inductive analysis of student responses to the various written and interview probes, we considered the possible sources of the difficulties. This was carried out in light of a model proposed by Schönborn *et al.* (2003 & 2002). These authors used an interview process to investigate the factors that affect student interaction with diagrams. Their empirical data supported 3 factors namely, the students' conceptual or prior knowledge and diagrammatic reasoning ability that they bring to the diagram, as well as the mode of representation or diagram itself (Schönborn *et al.*, 2003 & 2002). By investigating the possible sources of the difficulties, through in depth interviews with students, we were able to gain greater insight into their nature and apply this knowledge to the model of Schönborn *et al.* (2003 & 2002). As stated by these authors, it is not always possible to resolve the 3 factors that affect student interaction with diagrams, because like them, we often found that more than one of the factors may contribute to the source of a particular difficulty. Therefore, the aim of the model proposed by Schönborn *et al.* (2003 & 2002) is not to draw hard and fast conclusions about the source of a difficulty, but to determine whether the difficulty has more to do with, for example, the diagram than the reasoning factor or due to an interplay between 2 or more of the factors. Thus, by applying the results (student difficulties) obtained in the studies in Chapters 6 (Section 5.3.4) and 7 (Section 7.3.3) to this model (Schönborn *et al.*, 2003 & 2002), we aimed to view the difficulties in terms of their possible sources and, thereby, suggest possible remediation strategies that incorporated elements of diagram design and/or student instruction.

The rationale and critique of the range of methods used in this dissertation has been discussed in detail in this chapter. In Chapters 4- 7 that follow, the methods applicable to each of the studies will be highlighted while the current chapter will be referred where more detail is required.

CHAPTER 4

Expert Understanding of the Functional Features of Linear, Cyclic and Cascade Biochemical Processes

4.1 INTRODUCTION

What are biochemical processes? Cellular processes have been described by Kindfield (1992) as the dynamic changes that the chemical entities undergo in terms of their relative spatial arrangement and internal structure, constrained by the physical structures of the entities that are involved. The plethora of different biochemical processes in cells makes it necessary for biochemists to isolate sections of metabolic processes into more manageable functional units (e.g. the glycolytic pathway (see Chapter 6), the citric acid cycle and the complement system (Chapter 7)) that can be more readily researched, taught as well as learnt by students. In addition, as a means of further distinguishing one type of biochemical process from another, researchers have introduced terms such as “cyclic” which describes a wide variety of biochemical processes such as the citric acid or TCA (tricarboxylic acid), urea and glyoxylate cycles; and “cascade” which describes processes such as the complement pathways of the immune system, as well as the epinephrine, blood clotting and cyclic AMP cascades.

Why do we want to synthesise a meaning for the terms used to describe these different processes? Firstly, biochemical processes such as cycles and cascades have different physiological roles, which need to be clarified in textbooks. Secondly, although biochemistry textbooks use such terms to describe or identify certain biochemical processes, they often do not explain the nature and functional features of these biochemical processes. For example, what is a “cyclic” process, how do you distinguish between a cyclic process and other biochemical processes, and what is the importance of a cyclic process in our bodies? Thirdly, by finding the answers to questions such as these we aim to set up some distinguishing and generic characteristics of linear, cyclic and cascade biochemical processes that would constitute a “language” for metabolism researchers and textbook writers, thereby clarifying the meaning of these terms and facilitating

communication on this topic among biochemists. A “language of metabolism” would also help eliminate the difficulties students have with understanding the different biochemical processes and their functional roles. The identification and classification of some of these difficulties is the major objective of this dissertation.

Why did we use experts in this study? This is because it is the experts who research and define biochemical processes and who, therefore, write and compile the textbooks and lecture notes that students (the novices) use in the teaching and learning process. In performing this study, though, it was important that we remained cognisant of the fact that it is wrong to assume that novices will necessarily understand and visualise biochemical processes in the same manner as experts (Chapter 2, Section 2.3.3.2 & 2.3.3.4). This is because experts’ mental models differ from those of novices as they have more extensive prior knowledge and their knowledge bases are constructed differently to novices’ (Lowe, 1993b). Thus experts’ representations of problems (which are cognitive structures constructed by the problem-solver based on domain-related knowledge and its organisation) are superior to those of novices due to their extensive prior content knowledge (e.g. Chi *et al.*, 1981). So, before we could investigate any difficulties students had with understanding and visualising various biochemical processes (Chapters 5- 7), we wanted to ascertain up front, what experts thought constitutes the key functional features of linear, cyclic and cascade biochemical processes. This approach is in line with validity measures in Chapters 6 and 7, whereby experts’ opinions of our propositional statements were gathered (see Chapter 3, Section 3.3.5).

The research questions for this part of the study were as follows: (1) What is experts’ understanding of the distinguishing functional features (i.e. common to only 1 process) of linear, cyclic and cascade biochemical processes?; (2) Which features are generic to 2 or more biochemical processes?; and (3) Is there consensus among experts so that recommendation can be made for the description of these processes in textbooks and the literature?

4.2 METHODS

4.2.1 The Study Group

Twenty-three experts were e-mailed our questionnaire (Fig. 4.1 & Appendix A). Of these, 13 experts responded. All experts held doctoral degrees and senior lecture positions at both South African and international universities.

4.2.2 Analysis of Expert Responses

The analytic procedure is dealt with in detail in Chapter 3 (Section 3.3.7), and outlined in Chapters 6 and 7. All experts gave features in response to all the probes in Fig. 4.1.

4.2.3 Questionnaire: Design and Rationale

A questionnaire, comprising of the following 3 semi-focused probes, was designed and sent to 23 experts via e-mail, with an accompanying letter (Appendix A), in order to gather expert opinions on the distinguishing functional features of linear, cyclic and cascade biochemical processes:

- (a). What in your view are the distinguishing functional features of a linear process, e.g. the conversion of glucose to fructose 1,6 bisphosphate in glycolysis?

(b). What in your view are the distinguishing functional features of a cyclic process, e.g. the citric acid cycle?

(c). What in your view are the distinguishing functional features of a cascade process, e.g. the complement pathways of the immune system?

Figure 4.1: Examples of semi-focused type probes given to the experts in a questionnaire

Even though this dissertation considers 3 types of biochemical processes namely linear, cyclic and cascade processes, expert data was also collected on branched, parallel-coupled, “split”, shuttle and electron transport processes. The rationale behind supplying experts with examples of the different types of processes in the questionnaire (glycolysis, TCA or citric acid cycle and complement system) given to the experts (Fig. 4.1), was that students had previously studied examples of these processes in their lectures and this would, therefore, enable us to correlate expert and student data.

Two experts did not consider the glucose to fructose 1,6-bisphosphate reaction (see Fig. 6.1) supplied in the questionnaire (Fig. 4.1), to be an example of a linear process, because it contains branches due to its link to the pentose phosphate pathway. However, the TCA cycle also contains many branch points in which intermediates are common to other pathways, such as amino acid anabolism and catabolism. Furthermore, the TCA cycle is also composed of linear, parallel-coupled and branched biochemical processes. Therefore, if the path of one molecule was followed after entering the TCA cycle, most of the carbon atoms of this molecule may be fed into another pathway and therefore not complete the full “cycle”. Is this, therefore, a good motivation for not calling the TCA cycle a “cycle”? Similarly, coupled processes exist as part of many processes, including glycolysis and the TCA cycle. Clearly, linear, cyclic, and cascade processes may all contain one or more smaller processes, and this should be seen as a feature characteristic of each process. Indeed when terms like cyclic were first introduced they were used by people like Sir Hans Krebs (TCA or Krebs cycle) to describe cycles, although he was well aware they contained other processes.

In the following Results and Discussion section we synthesise and compare the opinions of various experts on the functional features of linear, cyclic and cascade biochemical processes.

4.3 RESULTS AND DISCUSSION

Inductive analysis (McMillan & Schumacher, 1993) of expert responses to the written probes (Fig. 4.1), led to the identification of a number of functional features for linear, cyclic and cascade biochemical processes. Synthesis of this data led to the grouping of these features (and 1 of the present author’s (AO)) into distinguishing features, (see Table 4.1 below), and generic functional features that are common to 2 or more biochemical processes (see Table 4.2).

Table 4.1: Distinguishing features of linear, cyclic and cascade biochemical processes based on expert responses (probes (a) – (c) of Fig. 4.1) and author's opinion (AO)

Distinguishing Features	Number of Experts/ 13
Linear processes	
1. They are diagrammatically represented in a straight line by convention	AO
2. They have the simplest pathway configuration	1
3. A substrate entering a linear process only passes through once before emerging	1
Cyclic processes	
1. They are diagrammatically represented in a circle by convention	AO
2. The beginning and end metabolite of the process are the same	5
3. A substrate moiety may undergo several cycles before emerging	3
Cascade processes	
1. They are diagrammatically represented in a step-wise form by convention	AO
2. The product of one reaction is the enzyme/ catalyst of the next reaction	6
3. They are involved in the amplification of a biological signal	9

Feature 1 for each process, suggested by the present author (indicated by author opinion (AO), in Table 4.1 above), constitutes one way in which linear, cascade and cyclic biochemical processes can be distinguished from each other. Although this distinguishing feature is not a functional feature of each process, textbooks conventionally depict cyclic processes as circles (e.g. diagram of the TCA cycle in Lehninger (2000, pg 587)); cascade processes in a step-wise form (e.g. diagram of the complement system in Roitt (1997, pg 24) as seen in Fig. 7.1, Chapter 7); and, linear processes as a straight line (see Fig. 4.2, below).



“A linear metabolic pathway. In this pathway, the reactant A is converted in five steps into the product F, with each step catalysed by an enzyme specific for that reaction.”

Figure 4.2: Representation of a linear metabolic process (Lehninger, 2000, pg 11)

The feature that linear reactions have the simplest pathway configuration (feature 2, see Table 4.1), was stated by 1 out of 13 experts. This expert did not elaborate on his/her list of linear features so, as indicated by the following quote, we were not able to establish whether this expert was referring to the conventional manner of linear process depiction or a more functional characteristic:

“Simplest pathway configuration...” [Response to (a) in Fig. 4.1]

No other experts made reference to the “structure” or manner of depiction of linear, cyclic or cascade processes.

The 3rd distinguishing functional feature of linear processes is that a substrate entering the linear process only passes through the process *once* before emerging. This point is illustrated by the following expert’s quote:

“Most of the carbon atoms that go in one end, come out of the other in one pass.” [Response to (a) in Fig. 4.1]

In contrast, the 1st and 2nd cyclic features (Table 4.1) i.e. that the initial reactant of a cyclic process is regenerated (stated by 5 experts), and that a substrate moiety may undergo several cycles before emerging (stated by 3 experts), may have emerged from some experts comparing cyclic and linear processes. This is illustrated by the following 2 expert’s quotes:

1. “... what makes cycles distinct from linear processes is that cycles involve a partial conservation of material (atoms and chemical groups), as the first substrate in the cycle is also the end product.” [Response to (b) in Fig. 4.1]

2. “Part of the molecule entering the cycle (say an individual carbon) may have to go around the cycle once or more, before emerging.” [Response to (b) in Fig. 4.1]

A major textbook, used by second year biochemistry students in a metabolism course, also considers the feature that cyclic intermediates are regenerated, to be central to the understanding of cyclic pathways. This is supported by the following 2 quotes:

1. "Some pathways are cyclic: one starting component of the pathway is regenerated in a series of reactions that converts another starting component into a product." (Lehninger, 2000, pg 487)
2. "We have now covered one complete turn of the citric acid cycle... at the end of the cycle a molecule of oxaloacetate was regenerated." (Lehninger, 2000, pg 579)

The idea of the regeneration of the starting molecule of the cycle (quote 1 above), constitutes an oversimplification, as there would be numerous molecules of the starting intermediate in a cycle only some of which might be the same molecule with the same atoms from the previous cycle. This is because atoms are continuously being added or removed from the cycle via other processes with which the cycle is linked. The conservation of atoms (see quote 2 above) is not unique to cyclic processes, as we know that there are many reducing and oxidising agents involved in parallel-coupled processes that are recycled between processes. However, within a cyclic process the atoms that are conserved are kept within the cyclic process and shall, therefore, be separated from molecules such as ATP and NADH etc.

In terms of the distinguishing functional features of cascade processes, the amplification of biological signals by cascade processes (see feature 3, Table 4.1) is well established. This feature is supported by the following expert quote and 2 further quotes taken from a biochemistry textbook pertaining to the epinephrine and glycogenolysis cascades, respectively:

1. "Cascades have the function of amplifying a signal. This is true in blood clotting, signal transduction, and the immune system." [Response to (c) in Fig. 4.1]
2. "Epinephrine cascade. Epinephrine triggers a series of reactions in hepatocytes in which catalysts activate catalysts, resulting in great amplification of the signal." (Lehninger, 2000, pg 453).
3. "We mentioned the control of glycogen breakdown, or glycogenolysis, as a particularly well-understood example of a regulatory cascade, a process in which the intensity of an initial regulatory signal is amplified many fold through a series of enzyme activations." (Mathews *et al.*, 2000, pg 474).

Of the 13 experts who responded to our questionnaire, 9 commented on the amplification of a signal by a cascade process (feature 3), which is higher than the number of experts who proposed any of the other features (Table 4.1). Feature 2 (Table 4.1), in which the product of a reaction acts as the enzyme or catalyst for the next reaction, was also a

popular feature for cascade processes amongst experts. This is illustrated by the following quote:

"A cascade involves a catalyst acting on (activating) another catalyst, which may activate another catalyst (and so on, until the desired number of levels). These catalysts are usually enzymes; the inactive forms are called zymogens, and they are usually activated by some covalent chemical reaction (e.g. proteolysis or de-phosphorylation)." [Response to (c) in Fig. 4.1]

By identifying the distinguishing features of linear, cyclic and cascade biochemical processes, i.e. the features that are characteristic only of the process in question, we believe we have effectively addressed research question number one of this study. In the next phase of our work, we addressed research question number 2, i.e. which features are generic or common to 2 or more of the 3 biochemical processes being studied? Experts' suggested features of linear, cyclic and cascade biochemical processes are presented in Table 4.2 below and discussed thereafter.

Table 4.2: Generic features of linear, cyclic and cascade biochemical processes based on expert responses (probes (a) – (c) of Fig. 4.1) and author's opinion (AO)

Feature		Number of Experts & Author Opinion (AO)		
		Linear	Cyclic	Cascade
1	The product of one reaction is the substrate of the next	5	AO	-
2	1 reactant (and more than 1 enzyme) forms 1 or more products	1	-	AO
3	1 reactant (and 1 enzyme) forms 1 product	5	-	AO
4	Each metabolite is chemically distinct from the previous	1	-	AO
5	Coupled reactions can be present in this process	3	AO	AO
6	No branches are present in this process	2		AO
7	Subject to (allosteric) control	5	3	AO
8	Overall process is irreversible	1	AO	AO
9	Process proceeds with an overall negative ΔG	1	AO	AO
10	Endergonic reactions are pulled or pushed by exergonic reactions	AO	1	AO
11	Intermediates fed into process from other processes	AO	5	AO
12	Produce intermediates for other processes	AO	5	AO
13	Consist of a specific number of molecules	AO	1	AO

14	Energy is invested in process	1	AO	AO
15	Rapid response/ product formation	1	AO	3
16	Process has catalytic nature	AO	3	AO

The 16 features given in Table 4.2, is clearly far from the exhaustive list of the functional features that characterise biochemical processes in general. There are countless other features that could be added including, for example, inhibiting one reaction will halt the entire process and every process has a net direction. However, what was pertinent to this study was an exclusive focus on the features provided by the 13 experts in our sample. In addition, author opinion (AO) was provided with respect to the relevance of each feature to the 3 processes as indicated in Table 4.2.

Only 2 features stated by some experts to be distinguishing of one process (response to probes in Fig. 4.1), were also stated by other experts to be distinguishing of another process, and were therefore classified as generic features. The first of these is feature 7 (Table 4.2), whereby 5 experts stated that linear, and 3 experts stated that cyclic processes, are subject to allosteric control as illustrated by the following 2 quotes, respectively:

1. "Properties of a linear pathway include mechanisms for regulating both flux and the size of the pools of the intermediate compounds, resulting from... and the action of allosteric and other rate modifiers." [Response to (a) in Fig. 4.1]
2. "The cycle will occur at a rate dependent on the *demand* for energy and such intermediates as long as there is sufficient substrate being *supplied* to the cycle." [Response to (b) in Fig. 4.1]

In addition to expert opinion, the author (indicated by AO) further classified cascade processes as being influenced by allosteric control (feature 7, Table 4.2), as described by experts in quote 1 and 2 directly above. Feature 15 (Table 4.2) was the second feature whereby 4 experts disagreed on which process was distinguished by this feature. One expert stated that linear, and 3 experts stated that cascade processes, ensured a rapid response or product formation as described by quotes 1 and 2 respectively:

1. "... amenable to high speed of conversion..." [Response to (a) in Fig. 4.1]
2. "Because of the catalytic nature of the activation process, very small amounts of initial factors suffice to trigger the cascade... which assures a large and rapid response to the trigger." [Response to (c) in Fig. 4.1]

Therefore, features 7 and 15 in Table 4.2 illustrate that there are in fact discrepancies in the opinions of experts with respect to the distinguishing functional features of various biochemical processes, a point that shall be revisited in Section 4.4.

Earlier, in Section 4.2.3 we introduced the idea that many biochemical processes consist of other smaller processes, for example, branched processes can occur in a cyclic process and coupled processes can occur in linear and cyclic processes. In this regard, 2 experts felt that a definition of linear processes should not include possible branching points. This is illustrated by feature 6 in Table 4.2, and by the following expert's remark:

"nor do I consider the segment of glycolysis from glucose to FBP [fructose 1,6-bisphosphate] as linear. In many instances, the pentose phosphate pathway interacts with this segment of glycolysis so there are additional issues of branching, regulation, and partitioning of intermediates." [Response to (a) in Fig. 4.1]

Another expert, however, did consider branches to be a possible part of linear processes as suggested by feature 2 (Table 4.2), in which s/he stated that one or more products could be formed:

"The function of a linear pathway is the conversion of a metabolite to one or more products, in stoichiometric proportions, by two or more enzymes." [Response to (a) in Fig. 4.1]

There is the possibility however that the above expert considered a sequence of reactions, of any length, to be a linear process in which case s/he may have been referring to the products of coupled reactions as being additional products of the overall linear process. By the same token, the above-quoted expert may have been indicating that branches could be part of linear processes, and further explanation is required before judgement on this particular expert's opinion is passed. When consulting a textbook used by second-year biochemistry students, in order to bring some clarity to the concept of branching in linear processes, the following excerpt was found:

"Some metabolic pathways are linear, and some are branched, yielding multiple useful end products from a single precursor or converting several starting materials into a single product." (Lehninger, 2000, pg 487)

The above quote, although seeming to separate linear and branched processes, is also ambiguous in terms of which process is forming the single or multiple end products. Another element of confusion, with respect to the above textbook quote, is the use of the term "pathways". All linear pathways must have branch points somewhere, and it may perhaps have been better if this textbook had used the term "process" and not "pathway" in this sentence. Furthermore, in terms of processes being composed of subsets of reactions, the 3rd feature of Table 4.2 is illustrated by the following expert's response:

"I see the linear process as little "sub-sections" of an overall reaction where a "subsection" consists of only one substrate reacting with one enzyme to form one product." [Response to (a) in Fig. 4.1]

The expert quoted above excluded both cyclic and cascade processes. This is because if only 1 reaction occurs, the same molecule in terms of molecular structure will not be

reformed therefore this feature could not be characteristic of cyclic processes (based on feature 2 of cyclic processes in Table 4.1). Also, in terms of cascade processes, feature 2 (see Table 4.1) is a distinguishing functional feature. However, if the above quoted expert's feature was also true for cascade processes, this would still mean that a cascade processes may contain subsets of linear reactions, reiterating a point made in Section 4.2.3 and by the above quoted expert, that processes may be composed of subsets of other processes. Three experts stated that coupled reactions could be present in a linear process (feature 5, Table 4.2) as indicated by the following quotes:

"...coupled reactions." [Response to (a) Fig. 4.1]

"Coupling to ATP hydrolysis..." [Response to (a) Fig. 4.1]

The above quotes further suggest that a definition of linear processes could include branches in which intermediates are gained or lost.

We suggest that feature 13 (as indicated by AO, Table 4.2) is a generic feature of linear, cyclic and cascade processes even though it was only suggested for cyclic processes as illustrated by the following expert's response:

"The process uses a specific number of molecules that accept an incoming component (molecule) at a certain point of the cycle. The component is passed on around the cycle either through conversion of the combined molecule (acceptor + component) into a new molecule or through transfer of the components to another molecule. ..." [Response to (b) in Fig. 4.1]

Therefore, feature 13 (Table 4.2) is an interesting feature, as it illustrates how some people might divide or compartmentalise processes into functional units of a specific number of molecules. By isolating segments of metabolic pathways into processes, people are therefore indirectly assigning functional "shapes" (e.g. circles) and set numbers of molecules into units and thereby labelling them as individual processes. This should, if the distinguishing functional features of each process are clarified, facilitate communication about these biochemical processes, an objective of the current study. However, If a network of biochemical processes are considered, then linear processes (which may be composed of smaller processes) may not be composed of a certain number of molecules but could be of any length, in contrast to cyclic and cascade processes, which might make this a distinguishing functional feature of linear processes under these conditions.

Thus, a number of interesting results and discussion points were found in experts' responses to the probes in Fig. 4.1. The overall results are summarised in the following section.

4.4 SUMMARY AND CONCLUSION

This chapter has addressed 3 research questions as stated in Section 4.1. In readdressing these questions, we consider the following comment by an expert from our sample:

“It is worth noting that none of the key stem words (linear, cyclic, coupled, cascade, branched, split, shuttle, transport) in the questions were invented specifically to describe biochemical processes. Rather they were taken over from the popular everyday English language of those who discovered them because these everyday words adequately described the biochemical processes concerned. They are therefore not usually considered worthy of specific definition as their meaning is essentially the one they have in ordinary language.”

The above quoted expert is of the opinion firstly, that the everyday words (e.g. linear, cyclic and cascade) adequately describe biochemical processes, and therefore, secondly, that they are not worthy of definition. Our results presented in Section 4.3 show the contrary, as summarised below.

Experts in our sample were asked for their distinguishing functional features of linear, cyclic and cascade biochemical processes and a number of features were obtained. From these features, we identified some features that were characteristic for only one process (see Table 4.1), thereby addressing our 1st research question, and others that were generic for at least one of the other 3 processes (see Table 4.2), which addressed our 2nd research question. Of the generic features in Table 4.2, there was a lack of consensus among experts and textbook authors as to which feature was characteristic of which process. This illustrates that the functional characteristics of these biochemical processes are not well established and that there is a need to clarify them, despite the above quoted expert's opinion. Our results also highlight how experts, to enhance communication, have divided metabolic networks into functional units or processes of defined length in terms of the number of reactions. However, this expert division of processes is only half complete because although we are aware of the functional features of, for example, the TCA cycle, we are not as sure about the functional features of cyclic processes in general.

None-the-less, the results of this study enable us to make various recommendations with respect to clear communication about linear, cyclic and cascade biochemical processes that may be used by teachers and researchers. The distinguishing and generic functional features of linear, cyclic and cascade processes summarised in Table 4.1 and 4.2, respectively, can be used to explain the nature of these processes to students and can be

incorporated into a “metabolism language” which will promote with more clarity what the nature of such processes are, and foster better communication with students and between researchers. In this regard, both the distinguishing (Table 4.1) and generic (Table 4.2) functional features of linear, cyclic and cascade biochemical processes, identified in this study, have already been assimilated into the second-year metabolism course, at the University of Natal, Pietermaritzburg (Anderson, 2003, pers. comm.). By moving towards synthesising meanings for the various types of metabolic processes, we can also gain an understanding into their physiological roles and importance. Therefore, through this investigation of linear, cyclic and cascade biochemical processes, the importance of extending this study to include data for other types of metabolic processes such as branches, splits, electron transport, parallel-coupled and shuttle biochemical processes, has been realised and preliminary data from experts has already been collected. The aim of this is to perform a more comprehensive study that might reveal more definitive characteristics of each type of biochemical process, and elaborate on the generic features of processes in general. It is possible, however, that we may not be able to draw up conclusive distinguishing characteristics for each type of biochemical process as there may always be at least one other process that shares these characteristics, meaning that a generic list might be more useful. The data gathered is none-the-less vital to our understanding of metabolism and the functioning of the cell and will contribute to the theory of biochemistry and how it is taught to students.

Furthermore, we suggest that the term “pathway” is a vague term and may incorporate any number of processes and, therefore, be of undefined length. Instead, we suggest that the term “process” replace “pathway” in the communication of metabolism as we have already taken the first steps to clarifying processes, in this study. In addition, in our opinion, a reaction may constitute a process, but a process is made up of more than one reaction. These meanings are assigned to the terms “reaction” and “process” used throughout the remainder of this thesis.

In summary, this study led us to draw the following major conclusions in light of our results:

1. There is a lack of clarity among experts on the meaning of the terms “linear”, “cyclic” and “cascade” despite the fact that they *are* frequently used to describe biochemical processes in textbooks and in the literature;

2. Two distinguishing functional features for each process were identified from experts' responses, to which we added one of our own (Table 4.1);
3. A number of generic features were also identified and there is a lack of consensus among experts as to which feature is characteristic of which process (Table 4.2);
4. Experts have divided metabolic systems up into processes of defined length (e.g. the TCA cycle) to enhance communication, but this division is only half complete since there is a lack of clarity with respect to the functional characteristics of these processes (e.g. cyclic processes in general);
5. We suggest that the term "pathway" is vague as it may be of undefined length and consist of many processes and, therefore, suggest that "process" be used instead;
6. There is a need to extend this study to describe and define the functional characteristics of other process types, including shuttles, coupled and branched processes;
7. We recommend that descriptions of the characteristic features of biochemical processes be incorporated into all metabolism curricula.

Now that we have identified experts' functional features of linear, cyclic and cascade biochemical processes, we return to the above quoted expert who had the following to say about formulating functional features of various biochemical processes (e.g. linear, cyclic and cascade) to aid students' understanding of them:

"...They are therefore not usually considered worthy of specific definition as their meaning is essentially the one they have in ordinary language... One has to ensure that students understand the basic idea(s) or concept(s) behind a biochemical process rather than having to memorize a specific definition so as to name or describe that process appropriately. The latter would tend to make matters worse rather than to help students whose English vocabulary may be restricted and thus would make the burden of learning the subject much heavier than it need be. An appreciation by students that these are normal everyday words being used to explain or describe biochemical processes is worthy of being cultivated by teachers of biochemistry..."

In this regard, the terms linear, cyclic and cascade *are* used in textbooks and literature, and coupled with the lack of clarity among experts presented in this chapter; we believe there is a need to clarify them. In the following chapter we investigate students' visualisation and understanding of linear, cyclic and cascade biochemical processes. It will therefore be interesting to note whether the above expert is correct in stating this assumption that everyday words (e.g. linear) are "not usually considered worthy of specific definition". Certainly, our results show otherwise in that "the basic idea(s) or concept(s) behind a biochemical process" are not well understood by experts. Furthermore, we do not have the

objective of formulating definitions for the everyday words (e.g. linear) to be rote learnt as suggested by the above expert. This chapter has served to clarify for us the meaning of linear, cyclic and cascade processes and, therefore, laid the groundwork for the subsequent studies on student visualisation of biochemical processes (Chapters 5- 7).

CHAPTER 5

Students' Models of Linear, Cyclic and Cascade Biochemical Processes Within Cells

5.1 INTRODUCTION

Kindfield (1992) describes subcellular processes as the dynamic changes that the entities involved in the process undergo in terms of relative spatial arrangement and internal structure, constrained by the physical structures of the entities that are involved. Thus, the conceptual understanding, and visualization of these processes would, therefore, be the construction of mental models of this sequence of dynamic changes in internal structure and of the relative spatial arrangement of the entities that are participants in the process, that are consistent with the current scientific models (Kindfield, 1993/ 1994 & 1992).

Alesandrini (1984) and Lowe (1997 & 1986) place flow diagrams, such as those of metabolic pathways, into an arbitrary or abstract diagram category where the diagrams do not resemble the objects or process that they are depicting, due to the extensive manipulations that have been done. They are nevertheless still conceptually and logically related to the real situation (Lowe, 1997). In contrast to realistic diagrams, flow diagrams do not show all the superficial detail and therefore concentrate rather on the deeper levels of content organisation (Lowe, 1997). This is because the content depicted by flow diagrams is often beyond our immediate or perceptual experience (Lowe, 1997). For example, diagrams representing biochemical processes in biochemistry depict the sequence of abstract events that constitute a biochemical process. In this regard, Hurt (1987) states that diagrams depicting analogies are often used when the content is abstract and are therefore functionally and literally different from realistic diagrams. Thus, the manner in which biochemical processes are depicted in textbooks can also be considered to be analogies as, for example, cyclic processes are conventionally depicted in a cyclic form and cascade processes in a step-wise form (see Fig. 6.1 of complement systems in Chapter 6). In all these cases, the analogy represents the *functional* properties of each of these types of processes, not the spatial or *structural* arrangement of metabolic intermediates

within cells (i.e. not how the pathways would actually look in cells when functioning if you had eyes that could view the sub-microscopic environment).

Britton and Wandersee (1997) state that “many processes in biology are better understood using diagrams and pictures”, while students have stated that they have better retention and form better mental models through the use of illustrations. Therefore, since flow diagrams depicting biochemical processes are a major tool for communicating the scientific model of biochemical processes, the generation, interpretation and use of diagrams forms part of students’ conceptual knowledge about the process (e.g. Kindfield, 1992). However, one of the cognitive disadvantages of using diagrams in biochemistry instruction is that the high degree of abstraction and the nature of the content depicted is often difficult to process (Lowe, 1994). Furthermore, the individual has to visualise an abstract biochemical process. As stated by Arnheim (1970, pg 315), “visual thinking calls, more broadly, for the ability to see visual shapes as images of the patterns of forces that underlie our existence- the functioning of minds, of bodies or machines, the structure of societies or ideas”.

Some of the difficulties students have with understanding biochemical processes may be attributed to students having no means through which to realistically visualise a process (Lowe, 1986). This is because “the depiction of the subject matter in scientific visuals is often not meant to be taken literally” (Lowe, 2000). At best, students have to rely on abstract or stylised diagrammatic representations of the sequences of events that make up the process. In terms of these diagrams, the perceptual organisation is influenced by the hierarchical structure of the diagram elements (Winn, 1991), which would be in the conventional textbook representation structures or spatial arrangements. This organisation of the symbolic diagram elements, i.e. molecules in our case, occurs involuntarily and depends on their proximity (Kosslyn, 1989), which is governed by the structures within which these biochemical processes are conventionally drawn. Furthermore, an individual’s prior knowledge and mental model of the content in the diagram determines whether an individual can make inferences from a diagram (Cheng *et al.*, 2001). Thus, if the individual has a limited content knowledge, everyday dynamics may be drawn on, resulting in the construction of an inadequate and inappropriate mental model (Lowe, 1999).

Two major research questions are addressed in this chapter. Firstly, what are students' mental models of biochemical processes within cells? Secondly, do, and if so, *how* do textbook representations of biochemical processes influence the mental models that students' construct?

5.2 METHODS

The general methods used in this thesis have been discussed in detail in Chapter 3. This section, therefore, deals only with the methods unique to the studies reported in this chapter.

5.2.1 The Study Group

The sample consisted of ninety-five, 2nd-year biochemistry students studying an integration of metabolism course. Thirty, 3rd-year biochemistry majors also participated in this study, and answered one of our probes ((a) in Fig. 5.1), as the second-year students had not yet studied a cascade process in their lectures.

5.2.2 Written and Interview Probes: Design and Rationale

Research Protocol Design and Rationale

We designed a 3-step research protocol to investigate students' mental models of biochemical processes within cells. In the first step, we used two semi-focused type probes (Fig. 5.1) to get students to reveal their conceptual understanding and mental models of cyclic and cascade processes. This information was then used, in step two, to inform the design of a series of diagrams (Fig. 5.2), and accompanying 2-tier multiple-choice probes, representing what we thought (from the results of step 1- see Table 5.1) were students' various mental models and visual characteristics of the biochemical processes. In this way, we hoped to clarify students' mental models, and related reasoning processes, in a "bi-directional manner"- from verbal to pictorial descriptions and then vice versa. Finally in step 3 we got students to both confirm what we suspected, from steps 1 and 2 were their mental models of the biochemical processes, and to reveal their visualization of such models *within cells*. This was done by interviewing students and asking them to verbally

describe, as well as construct their own diagrams, of how they visualised the processes occurring within cells. In this respect, the research design involved triangulation (see Section 3.3.5 of Methods Chapter) involving 3 different approaches for obtaining data about students' mental models. The details of the probes and methods used in this protocol, and the rationale behind them, are given below.

Probe Design and Rationale:

Step 1: The initial information on students' conceptual understanding and mental models of biochemical processes, was obtained by giving 2 semi-focused written probes (Fig. 5.1) to the students at two-week intervals. This was after the second-year students had been taught the TCA cycle, and the third-year students had been taught the complement system (see Chapter 6) in their respective biochemistry courses. A free-response type question about linear processes was not given to students in the sample, as we were not sure at this stage of the investigation whether the students had studied an example of a "linear" process in their lectures. The third year students were given a coloured copy of the complement system diagram, as seen in Fig. 7.1 (Chapter 7), while the second year students were provided with a coloured overhead transparency of the TCA cycle (Karp, 1999, pg 189). The responses to the questions were analysed as described in Section 3.3.7 (Chapter 3) and summarised in Table 5.1 for use in step 2 below.

<p>Use the diagram provided to answer the question that follows.</p> <p>(a). The complement system is an example of a cascade process. Discuss why you think it is a cascade.</p> <p>(b). The tricarboxylic acid (TCA) cycle is an example of a cyclic process. Discuss why you think it is a cycle.</p>
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Figure 5.1: Semi-focused type probes given to the third and second year students, respectively

Step 2: In step 2, we used the results from step 1 (summarised in Table 5.1, below) to inform the design of a series of diagrams (see Fig. 5.2, below) and accompanying 2-tier multiple-choice probes (Treagust, 1988), to gain greater insight into students' mental

models and visual characteristics of the biochemical processes. The rationale behind the design of these diagrams is discussed below.

Table 5.1: Overview of student responses to the probes in Fig. 5.1 (step 1) and the corresponding diagrams (Fig. 5.2) and probes designed in step 2 to further investigate each response.

Step 1		Step 2	
Probe (Fig. 5.1)	Student response(s)	Diagram Number	Mode of Representation
Cascade processes (see probe (c), Fig. 5.2)			
(a)	-The product of one reaction is the enzyme or catalyst for the next reaction. -Literal interpretation: cascade processes occur in a step-wise or cascade fashion <i>in vivo</i> .	10	Conventional
		11	
Cyclic processes (see probe (b), Fig. 5.2)			
(b)	-The starting product is also the end product of the cycle (vice versa). -Literal interpretation: cyclic processes occur in circles <i>in vivo</i> .	6	Conventional
	-Literal interpretation: cyclic processes occur in circles <i>in vivo</i> . -Literal interpretation: linear processes occur in straight lines <i>in vivo</i> .	7	Unconventional
Linear processes (see probe (a), Fig. 5.2)			
(a) and (b)	- The product of one reaction is the substrate for the next reaction.	1	Conventional
		2	
		3	
	- Literal interpretation: linear processes occur in straight lines <i>in vivo</i> (based on students' literal interpretation of cyclic and cascade processes)	4	Unconventional
		5	
		8	
		9	

Although not indicated in Fig. 5.2 below, probes (a) – (c) had 5 bullets under each question to encourage the students to motivate for their selection of each diagram with more than one reason. The diagrams in Fig. 5.2, below, filled an A4 size page when given to the students. To assist the reader we have included the classification of each process (e.g. as linear) next to each figure, while an alternative copy of Fig. 5.2 without the processes labelled can also be found in Appendix B.

Use the diagram sheet provided below to answer the following questions

(a). Which diagram(s) represent(s) linear process(es)?

Circle your choice(s): 1 2 3 4 5 6 7 8 9 10 11

I made the above choice(s) because **linear** processes have the following characteristics:

(b). Which diagram(s) represent(s) cyclic process(es)?

Circle your choice(s): 1 2 3 4 5 6 7 8 9 10 11

I made the above choice(s) because **cyclic** processes have the following characteristics:

(c). Which diagram(s) represent(s) cascade process(es)?

Circle your choice(s): 1 2 3 4 5 6 7 8 9 10 11

I made the above choice(s) because **cascade** processes have the following characteristics:

1-Linear 2-Linear 3-Linear 4-Linear 5-Linear 6-Cycle 7-Cycle 8-Linear 9-Linear 10-Cascade 11-Cascade

KEY:
A cellular membrane is represented by:

Figure 5.2: Multiple-choice type probes given to second year students to investigate any literal interpretations of representations of biochemical processes

Rationale for the design of the diagrams in Fig. 5.2

From analysis of the students' responses we obtained from probes (a) and (b) in Fig. 5.1 (see Table 5.1), we were able to pick out features that students used to characterise cascade and cyclic processes and thereby design the diagrams used in the probe in Fig. 5.2. These diagrams were kept simple on purpose, without including details of intermediates, to facilitate student identification of the processes based on their perceptual / structural/ functional features, without additional aspects that may complicate the diagram. This is because the working memory can only deal with a certain amount of information at any one time, and we also therefore hoped to decrease cognitive load (Lowe, 2001, pers. comm.). The diagrams (Fig. 5.2) were specially designed to be, what we termed, either "conventional" or "unconventional" modes of representation or spatial arrangements. In this regard, we termed conventional representations as those that the student is used to seeing in textbooks or lecture notes. For example, linear reactions or processes are usually drawn in a straight line or linear form, cyclic processes in a cyclic or circular form, and cascade processes in a step-wise form. Thus, the unconventional representations are, therefore, those depictions that are different to typical and common forms. For example, linear processes drawn in non-linear form such as in a semi-circle or step-wise form and cyclic processes drawn in a linear or straight-line form. The rationale behind the use of both conventional and unconventional modes of representing the processes, was to gain greater insight into the literal nature of students' interpretations of diagrams of processes, revealed in step 1 (see Table 5.1), and, therefore, to understand students' mental models of such processes in cells. In other words, to see if students would identify a cyclic process only if it was drawn in a cyclical format, not if it was represented in a linear format. If so, this would suggest that the student was identifying the cyclical process based on a literal interpretation (LI) of the diagram in which the superficial characteristics, rather than the functional characteristics of the process were used to understand the process. This is further explained below in terms of the design of each diagram.

Diagrams 10 and 11 (see Fig. 5.2; Table 5.1) are conventional stepwise representations of actual cascade processes, as seen in textbooks. Their design was based on correct and incorrect student responses (Table 5.1) to probe (a), Fig. 5.1, that the product of one reaction is the enzyme for the next reaction, and that cascade processes occur in a step-wise or cascade fashion *in vivo*, respectively. Diagram 4 also shows a stepwise-type of representation, however it represents a linear process, rather than a cascade process. It was

designed from students' correct and incorrect responses (Table 5.1) to probes (a) and (b) of Fig. 5.1, regarding linear processes, in that the product of one reaction is the substrate for the next reaction, and that all linear processes occur in "straight lines" *in vivo*. The rationale behind this diagram was that if the students were interpreting diagrams literally, they would think that diagram 4 represented a cascade process (and indicate this in answering probe (c), Fig. 5.2). However, if they understood the functional characteristics of linear processes they would recognise diagram 4 as a linear process (and indicate this in answering probe (a), Fig. 5.2), just represented as a stepwise, conventional cascade type representation.

A similar rationale was used to design the other diagrams. With respect to cyclic processes, students' correct and incorrect responses to probe (b) in Fig. 5.1, led to the design of diagrams 6, 7, 8 and 9. Diagram 6 represents a conventionally depicted cyclic process drawn in a circle. In contrast, although diagram 8 is also a conventional method of depicting a cyclic process, there is a branch at "F" and the end product of the cycle is therefore not the starting product of the reaction, and diagram 8 is therefore not a cyclic process. Similarly, diagram 9 is another conventional method of depicting a cyclic process but there is no reaction between "E" and "D" and it is therefore a linear process. Diagram 7 was designed from students' correct and incorrect responses, in that the beginning and end metabolite of a cyclic process are the same, and that cyclic processes occur in "circles". We wanted to test whether students were looking at the superficial depiction of a cyclic process or whether they were considering the deeper, functional aspects of a cycle.

In terms of students' identification of linear processes, there is some overlap with the diagrams that were constructed from students' responses to probes (a) and (b) in Fig. 5.1. The conventional manner of representing a cascade process, but incorporating the students' difficulty that the product of one reaction is the substrate of the next (i.e. diagram 4, of the probe in Fig. 5.2), would constitute an unconventional manner of representing a linear process. By the same token, diagrams 8 and 9 are also unconventional methods of depicting linear processes, although there is a branch at "E" in diagram 8. Diagram 7, which is an unconventional method of depicting a cyclic process, would therefore be a test to determine whether students were using superficial linear characteristics in identifying diagram 7 as a linear process because it is drawn in a straight line. Similarly, diagram 5 was also designed in the same light, as we wanted to test whether students would recognise

that a linear process is not necessarily confined to a single cellular compartment, nor is it required to be drawn in a straight line. Thus, we designed an unconventional representation in which the linear process is depicted as 3 sides of a rectangle, straddling a biomembrane. Conventional representations of linear processes were also designed and can be seen in diagrams 1, 2 and 3 (two linear processes occurring on either side of a membrane).

Step 3: In step 3, we wanted to specifically probe students' mental models of biochemical processes, in other words, as they would 'see' them within cells. To investigate this we made use of interviews (verbal data) and student-generated diagrams (non-verbal data). We wanted to collect both verbal and non-verbal data as it is believed that there is a difference in an individual's mental and expressed models, which means that an individual should be asked to elicit more than one of their expressed models (Reiss & Tunnicliffe, 2001) in order to come closest to revealing their 'true' mental model (see Chapter 3, Fig. 3.1). Student-generated diagrams were also used in the interviews in order to overcome any distortions that may arise when students try to communicate their visual information into the spoken word (Lowe, 1993b). This non-verbal data is also believed to contribute to our understanding of how mental models are constructed and how reasoning is executed (e.g. Gobert & Clement, 1999 & Lowe, 1993b & 1991) whereas problem-solving tasks with the diagrams are believed to expose students' mental models (Kindfield, 1992).

Once all the responses to the written probes (seen in Figs.5.1 & 5.2) had been analysed, interviews were conducted with 15 student volunteers from the second-year biochemistry course. During the interviews, the students were provided with an information sheet containing Diagrams 1-11 (Fig. 5.2) as well as the following keywords: Linear, Cyclic, Cascade, Branched, Parallel-coupled and Shuttle, so that they could select a word to describe a process. The second year students had covered examples of each of these types of processes described by the keywords in their biochemistry lectures. Students were also provided with coloured pens and paper for the construction of student-generated diagrams. The interviews were videotaped, transcribed and analysed. A more detailed account of the interview procedure used in all chapters of this thesis can be found in Section 3.3.4 (Chapter 3, Methods).

5.3 RESULTS AND DISCUSSION

We used the 3-step research protocol (Section 5.2.2) to address our two research questions namely, what are students' mental models of biochemical processes within cells, and, do textbook representations of biochemical processes influence the mental models that students' construct? In this section we attempt to answer the two research questions in the light of the results obtained from the methods used in our 3-step research protocol, which were designed to progressively gain greater insight into the nature of students' mental models of biochemical processes within cells. The results obtained in each step are presented and discussed in sections 5.3.1, 5.3.2 and 5.3.3, respectively.

5.3.1 Preliminary Evidence for Students' Models of Biochemical Processes (Step 1)

Student responses to the probes in Fig. 5.1, yielded preliminary evidence for the nature of students' mental models of biochemical processes. The following are typical quotes that illustrate these findings:

1. "It's a cascade effect because each molecule seems to fall into the next. For example: C3 → (convertase) → C3a & C3b; which in turn will activate/ deactivate processes." (Response to probe (a))
2. "Because one reaction flows with the next...one depends on the other. They follow a sequenced chain of reactions." (Response to probe (a))
3. "... kind of domino effect, cascade → like clotting cascade." (Response to probe (a))
4. "The rxns [reactions] follow a cyclic path, thus starting the circle at oxaloacetate and ending at oxaloacetate. The pathway however starts at pyruvate. Thus the rxns [reactions] follow a cyclic path, and moves in a circle." (Response to probe (b))
5. "The reaction occurs in a circle, the reactants reacts [react] to form a product and the product then becomes the reactants (or substrate) for the reaction. It is an ongoing process..." (Response to probe (b))
6. "... It is a continuous process. It is a closed circle of reactions and not a straight line of reactions." (Response to probe (b))

The above student responses (also summarised in Table 5.1) suggest that some students might be basing the construction of their mental models of biochemical processes on a "direct copy", or literal interpretation (LI) of conventional textbook representations of such processes. In other words, it appears that they were formulating a mental model of the *structure* or *spatial arrangement* of such processes in cells (i.e. how the molecules, reactions and pathways would be physically distributed and actually look like if you could see them occurring in cells), from a textbook diagram designed to purely represent the

functional, not the structural features of such processes (i.e. to show what intermediate reacts with what substrate to form which product in which sequence). Support for this suggestion is found in, for example, quotes numbered 1-3 that refer to a cascade that can be seen literally e.g. a water cascade, and quotes 4-6 illustrate how these students literally refer to the circular structure of the reactions.

The above findings are consistent with those of Humphries and Bruce (1989) who state that the first stage of diagram processing is at the visiospatial level. Students would, therefore, have to semantically classify the figures by relating the diagram elements to their function, seen as the second stage of visual processing (Humphries & Bruce, 1989). This is carried out in order to determine the nature of the process, as linear, cyclic or cascade.

5.3.2 Students' Classification of Biochemical Processes Based on Literal Interpretation (LI) (Step 2)

Step 2 of our research protocol (Section 5.2.2), attempted to gain greater insight into what appeared, from step 1, to be students' inappropriate mental models of biochemical processes based on LI of textbook diagrams. To achieve this, we investigated students' use of LI to classify various representations of linear, cyclic and cascade processes. This involved using the results from step 1 (Table 5.1) to inform the design of a series of diagrams, and accompanying 2-tier multiple-choice probes (see Fig. 5.2) (Treagust, 1988), that students would classify in terms of the processes they thought the diagrams were representing.

Table 5.2 provides a summary of the number of students who classified the linear, cyclic and cascade processes on the basis of their LI of the supplied non-conventional representations of the processes (Fig. 5.2).

Table 5.2: Number of students who used literal interpretation (LI) to classify non-conventional representations (Fig. 5.2) of biochemical processes

Diagram number	No. of students who chose process/ total no. students	Incidence (%)
Students classifying non-linear process as linear		
7	44/81	54.3
Students classifying non-cyclic processes as cyclic		
8	73/83	88.0
9	17/66	25.8
Students classifying non-cascade process as cascade		
4	19/86	22.1

Although Table 5.2 above shows the number of students who classified a process based on the literal manner in which it was drawn, the number of students who actually used LI in words in their explanations of why they chose the particular representations (probes in Fig. 5.2), was found to be 18.9% (18/95) of students for each of the linear and cyclic processes (probes (a) and (b) in Fig. 5.2), and 12.6% (12/95) of students for cascade processes (probe (c) in Fig. 5.2). The total number of students selecting any of the diagrams in Table 5.2 in response to probes (a), (b) or (c) (Fig. 5.2) remained fairly constant except in the case of diagram 9 where only 66 students classified it. This may have been due to students being unsure of the nature of this cyclic-drawn linear process, and this consequently may have been responsible for the large discrepancy in the resulting incidence of students classifying diagrams 8 and 9 (Fig. 5.2) as cyclic (see Table 5.2).

5.3.2.1 Literal Interpretation (LI) of Linear Representations of Biochemical Processes

Students' classification of the diagrams in Fig. 5.2 revealed strong evidence for students' LI of representations. For example, as seen in Table 5.2, over half the students (54.3%, 44/81) classified diagram 7, which is a cyclic process, as a linear process. The following quotes in response to probe (a) in Fig. 5.2 are illustrative of students' LI difficulties. Student choices of diagrams are indicated in parenthesis after each quote.

1. "Linear processes occur in a straight line. Linear processes occur at 180°C [180°] in any direction... and occur vertically or horizontally." (Choice(s) 1, 2)
2. "It occurs in a straight line." (Choice(s) 1, 2, 3, 4, 7, 9)
3. "Even though some of the reactions are reversible and some not, that doesn't change the fact that the processes are linear. The reactions are in a line." (Choice(s) 1, 2, 7)
4. "This process does not go in a circular fashion where the final end product starts the reaction again... this process is carried out in a line." (Choice(s) 1, 2, 4, 7, 9)

As seen from the above quotes, these students state that linear processes occur in straight lines. Since the conventional method of depicting linear processes in textbooks is as a linear representation, this may be leading students into thinking linear processes occur in straight lines *in vivo*, although it was not completely clear at this stage of the investigation. Also the fact that all the above students (1-4) chose at least two of the conventional representations of linear processes, namely diagrams 1, 2 and 3 (Fig. 5.2), constituted further evidence that these students were using the visual feature, that linear processes are represented in a straight line, to classify linear processes. Further evidence for such LI of linear processes was found in the fact that all, except one student, recorded the LI feature as their first characteristic of linear processes, in their responses to probe (a) in Fig. 5.2. By contrast, students 2 and 4, who classified the unconventional representations of linear diagrams 4 and 9 (Fig. 5.2) as linear, may have been thinking more deeply about the functional characteristics of linear processes i.e. that the starting and end product are not the same. Thus, this may show that these students (2 & 4) have conflicting mental models since they express LI but also chose diagrams 4 and 9, which are not straight-line representations.

The following 2 interview extracts further support the finding that some students classify linear biochemical processes as linear because they are drawn or represented in straight lines:

I: What do you think process number 7 is [points to diagram 7]?

S: um, that would be linear as well because it's going in a straight line, although this one is not only going in one direction this one can occur in the reverse direction but I think it is also linear

As expected, the above student did not classify the unconventional linear representations (diagrams 4 and 9) as linear during this interview, probably because they were not drawn in a linear format.

Furthermore, the following student also used the same reasoning (LI) to incorrectly classify diagram 7 as linear, but correctly classified diagram 9 as linear, even though it was depicted in a circular format. This was because s/he realised that "D" and "E" are not joined by an arrow:

I: What type of process is number 7 [points to diagram 7]?

S: I think it's linear, with the reversible reactions like between B and C and between C and D and between D and E, so it forms a straight line

I: and what about process 9 [points to diagram 9]?

S: I'd say it's not circular since D and E are not joined they are, ja they are not joined so it's only from E backwards to D there's no link here

I: so what type of process do you think it is?

S: mmm, it can be linear in other words not a straight line but linear.

In summary, the above evidence suggests that many students were classifying biochemical processes as linear based on their LI of diagrammatic representations that were linear in terms of their spatial arrangement. This, in turn, suggested that students' mental models of linear processes might have the appearance of reactions occurring in straight lines within cells. This possibility was further investigated in the study reported in Section 5.3.3. However, the results also showed that some students did state other features of linear processes that may be why some students were found to classify unconventional linear processes representations (Table 5.1) as linear.

5.3.2.2 Literal Interpretation (LI) of Cyclic Representations of Biochemical Processes

In response to probe (b) in Fig. 5.2, we found that 18 out of a total of 95 students (18.9%) revealed that LI was one of their reasons for classifying any combination of the diagrams found in the probe in Fig. 5.2 as cyclic. In this regard, the following quotes illustrate the LI feature in response to probe (b) in Fig. 5.2, and are examples of the 9 out of 18 students (50%) who chose diagrams 6 and 8, 6 out of 18 students (33.3%) who in addition also chose diagram 9, and 2 out of 18 (11.1%) students who chose diagram 7 as cyclic process representations:

1. "The reactions must be in a circle or go round and round." (Choice(s) 6, 8)
 2. "The pathway/ series of reactions forms a complete circular structure." (Choice(s) 6, 8)
 3. "Move in a cyclic manner i.e. not in a straight line." (Choice(s) 6, 8, 9)
 4. "Occurs in a circular movement." (Choice(s) 6, 8, 9)
 5. "It occurs as a cycle and is continuous... occurs in a circular motion i.e. not linear." (Choice(s) 7, 8)
- "Occur in a circular direction ∴ has no beginning or end." (Choice(s) 6, 7, 8)

In addition to the above quotes, another student also used LI and only classified diagram 8 as a cyclic process, the reason for which is unknown. Quotes 1 and 2 above show how students who stated the LI feature and chose diagrams 6 and 8 (see Fig. 5.2), consider this to be a primary feature of cyclic processes as they did not consider any of the other cyclic processes (see Table 5.1) in their choice of diagrams representing cyclic processes.

However, these students did mention other features of cyclic processes, but these features apparently did not lead them to consider whether any of the other diagrams in the probe in Fig. 5.2 were of a cyclic nature. In addition, the above quotes show how some students may have been careless about choosing diagrams 8 (quotes 3- 6) and 9 (quotes 3 & 4) as cyclic because they may have seen the circular shape, but not considered the arrows in these diagrams as there is no arrow between “E” and “D” in diagram 9, and diagram 8 is not a full circle in terms of the arrows. Furthermore, the above quotes illustrated how these students may have thought that cyclic processes proceeded in an actual circular shape *in vivo* and this may be a reason why some students quoted above (quotes 1-4) did not choose diagram 7, the unconventional cyclic process representation as cyclic. However, two students (quotes 5 & 6) did choose diagram 7 (Fig. 5.2) and also used the feature that the beginning metabolite or reactant of a cyclic process is reformed (in their responses to probe (b) in Fig. 5.2), which may be why they considered diagram 7 to be a representation of a cyclic process. This suggests that although students may use LI to classify representations of biochemical processes, they may not actually think that these processes occur within these spatial arrangements as conventionally depicted in textbooks. This is discussed further in Section 5.3.3. In other words, students may hold two conflicting models of such processes, with the one or the other being used depending on the context or nature of the question.

When the following student was asked to classify diagrams 8 and 9 (from Fig. 5.2) in an interview, although s/he considers that the product of one reaction is the reactant of the next reaction, s/he doesn't mention that the beginning and end metabolite of a cyclic process are the same. As a consequence, the LI feature is held strongly by this student, illustrating the mixed or conflicting nature of his/ her mental model:

I: ... and what about number 8 [points to diagram 8]?

S: that's cyclic because it's going in a circle around, um and the reaction, the products of the one forms the reactant of the next so they each, each one is necessary for the next one to occur.

I: and number 9 [points to diagram 9]?

S: mm, it's quite hard because it's not a full cyclic one but I think that's also cyclic except it just isn't complete, but I think, ja, no wait hold on, no I think this one, no I don't know I think it's cyclic.

During probing the above student about diagram 9, we can see that this student is aware that there is no arrow connecting “D” and “E” but nevertheless holds the LI feature very strongly and therefore classifies diagram 9 as cyclic and not linear.

In the same respect, the next student also shows the LI feature as s/he classifies diagram 8 (in Fig. 5.2) as cyclic because it goes round and round and does not consider the arrows in the diagram:

I: ...and how about process number 8 [points to diagram 8]?

S: that's a cyclic process

I: and what told you that that was cyclic?

S: because each reaction produces a product which goes round and there's no end it just keeps going round.

In contrast, the next interview extract shows how the following student used the feature that the initial substrate in a cyclic process is reformed, and the LI feature, to initially classify diagram 8 (from Fig. 5.2) as cyclic. However, this student then considers the arrows in the diagram and changes her mind and classifies diagram 8 as linear:

I: ... and what about diagram number 8 [points to diagram 8]?

S: mm, that would be cyclic, yes it would be cyclic

I: so how do you decide whether it's a cyclic pathway?

S: the beginning prod.. substrate is reformed, it occurs in a circle, a circular form. That's what I think, cyclic, but however... just hold on let me have a look at this [student studies diagram 8] E doesn't form F, so it can't be cyclic

I: ok, so what do you think it is?

S: it can't be cyclic... it's not cyclic it's not in an entire circle because one reaction could halt the process like E to F halt the process because E didn't form F. And, so F to A is another reaction completely it's not dependant on the reaction of A to B or B to C or you know what I mean. And it's also linear as it's written here, linear.

This section revealed some interesting results that showed that students do not always consider the arrows in a diagram. This may have led some students to incorrectly classify biochemical process diagrams. Some students, although using LI to classify figures, may not actually think that these processes occur within these structures *in vivo*. In this regard, some students' responses suggested that mixed mental models of cyclic processes may exist when students LI and any other feature of cyclic processes used by the students do not correspond. In the same manner, LI was shown to be a characteristic that was used by some students to classify diagrams depicting biochemical process as either linear or cyclic. This same pattern was also identified in cases where students were required to classify diagrams depicting cascade biochemical processes. An extension of this is dealt with in the section that follows.

5.3.2.3 Literal Interpretation (LI) of Cascade-like Representations of Biochemical Processes

In response to probe (c) in Fig. 5.2, 12 students (12/95, 12.6%) showed LI in words when classifying various processes as cascade. The following quotes illustrate this finding:

1. "Exhibit properties similar to a cascade." (Choice(s) 10, 11)
2. "They also take place in a step like formation so that they look like a cascade." (Choice(s) 10, 11)
3. "A cascade is sort of like a step patterned reaction, \therefore I chose reaction [diagrams] 4, 10 and 11, because they seem to have that pattern." (Choice(s) 4, 10, 11)
4. "When drawn it looks like a "waterfall". Looks like steps because reactions require "steps" to move from one rxn [reaction] to the next." (Choice(s) 4, 10, 11)

The above quotes show how these students literally interpreted the meaning of the word "cascade" and applied it to how they thought a cascade process looks on paper. Whether these students think these processes look like everyday cascades when occurring in the cell, will be dealt with in Section 5.3.3. From the above quotes, students 1 and 2 chose the conventional cascade representations but did not choose diagram 4 (see Fig. 5.2), probably because they realised that this was a linear representation based on other features in their answers. The latter two students (quotes 3 and 4) chose diagram 4 (see Fig. 5.2) as an example of a cascade process representation, instead of seeing that it represents a linear process drawn in a step-wise or cascade form.

Of the 12 students who showed this LI feature in response to probe (c) in Fig. 5.2, 4 students (4/12, 33.3%) chose diagrams 10 and 11 as examples of cascade processes and correctly classified diagram 4 as linear, therefore illustrating that these students may use a combination of features and not only LI to classify processes. A further 5 students (5/12, 41.7%) chose diagrams 4, 10 and 11 as cascade process representations, possibly due to LI. Furthermore, 2 students (2/12, 16.7%) only classified diagrams 10 and 11 as cascade representations (and did not classify diagram 4), while 1 student (1/12, 8.3%) only classified diagrams 4 and 11 as cascade representations. Therefore, a total of 6 students of the 12 (50%) who used this LI feature in their response to probe (c) in Fig. 5.2 classified diagram 4 as a cascade process representation. The reasons for these students incorrectly classifying diagram 4 as a cascade process may not be solely due to the LI feature, as some of these students also thought that in a cascade process, the product of one reaction is the substrate of the next, a feature found to be stated by 14.7% (14/95) of students in response to probe (c) in Fig. 5.2.

The following interview extract illustrates how some students use LI to classify the conventional cascade process representation:

I: and what about number 10 [points to diagram 10 in Fig. 5.2]?

S: I think it's the cascade since I mentioned earlier that cascade forms, they are in a step form so each one looks like that.

Furthermore, the next student also illustrates how LI may override other characteristics when students classify processes. Although this student knows that diagram 10 (in Fig. 5.2) is a cascade, s/he classifies it as a linear and cascade process because some reactions are in a "straight line":

I: ok, let's take a look at some examples of processes now, you can use these keywords [points to sheet of keywords given to the students, see Methods Section 5.2.2] to describe what type of process they are [shows student diagram sheet in Fig. 5.2]. What type of process do you think number 10 is [points to diagram 10 in Fig. 5.2]?

S: ... mmm it's a linear, linear and... [mumbles] it's [a] linear and cascade reaction

I: now why do you think it's a linear and cascade reaction?

S: mm, it's linear because it's product is being formed in a straight line reaction, and cascade this part is being formed before this part

I: ok so A and B are formed before C and D?

S: yes.

Thus, the emergence and recurrence of LI has been shown with respect to various student responses, about linear, cyclic and cascade biochemical processes, to the written probes in Figs. 5.1 and 5.2, as well as some interview extracts. Evidence was also gathered to indicate that LI might not be the sole reason for students classifying a non-cascade process drawn in a step-like form. This was because some students incorrectly thought that the product of one reaction in a cascade is the substrate of the next reaction. However, in cascade processes, the product of 1 reaction is the enzyme or catalyst of the next reaction (Chapter 4, Table 4.1).

In summary, in terms of linear cyclic and cascade biochemical processes, preliminary evidence was collected on students' mental models of these processes. Apart from the scientifically acceptable model, in which students link the diagrams to their knowledge of the functional characteristics of the 3 processes, there is the incorrect mental model resulting from the LI of biochemical processes, as a result of the spatial arrangement depicted in conventional representations. Students with the LI difficulty interpret

conventional representations as the functional representation of biochemical processes. In addition, some students showed that they may hold a combination of various conflicting or mixed mental models based on student understanding of other features of the processes. Investigations into students' mixed mental models, and whether or not students stating this LI feature think that biochemical processes occur in the cell in the same "spatial arrangements" or "structures" as they are represented in textbooks, will now be dealt with in the following section.

5.3.3 Students' Models of Biochemical Processes *In Vivo* (Step 3)

Preliminary evidence was provided in the studies reported in sections 5.3.1 and 5.3.2 of how students appear to hold various mental models of biochemical processes. These mental models stem primarily from students' use of LI to classify various representations of linear, cyclic and cascade processes (Sections 5.3.2.1- 5.3.2.3 respectively). In this section, we report on studies aimed at delving deeper into the nature of these mental models in terms of the occurrence of biochemical processes within cells. This was done by means of interviews with 15 student volunteers from the second year biochemistry sample (see step 3, Section 5.2.2). Students were encouraged to use letters to represent molecules in their diagrammatic representations of biochemical processes, to decrease cognitive load as the working memory can only deal with a certain amount of information at any one time (Lowe, 2001, pers. comm.). The variety of student responses gathered from interviews will now be considered.

In the previous sections on linear, cyclic and cascade processes (5.3.1 & 5.3.2), we were not sure whether all students who used LI to interpret diagrams, actually thought that the biochemical processes occurred in their conventional textbook representation "spatial arrangements" or "structures", or whether they had just become used to seeing these representations and therefore had built LI into how they identified these different processes. The following sets of interview extracts, by focussing on students' mental models of the molecules moving and reacting as part of biochemical processes in cells, showed how some students in the second year sample do think that molecules participating in linear processes occur in a "straight line", cyclic in a "circle" and cascade in a "step-wise" form inside the actual cell itself.

To investigate the above issues, we commenced the interviews by probing generally about students' mental models of molecules moving and reacting in cells as part of biochemical processes. The following 2 interview quotes are typical of the responses obtained:

I: What do you think the molecules would look like, the way they'd be moving and reacting?

S: Oh, the molecules, ja it would be like a very um, like structured it wouldn't be like roaming around bumping into each other I think it would be like um efficient you know like a streamlined thing happening, and the exchange between different organelles.

The above student's response supports the LI model of thinking whereby this student visualises biochemical processes as having a structure, and does not visualise a dynamic exchange system, whereby chemical reactions are occurring through favourable colliding of reacting molecules.

In contrast to the above student, the first part of the next interview extract shows how another student creatively described the fluid nature of the cell using an analogy of water in a glass, but described the molecules found within a cell as having shapes such as "balls" and "squares", which may come from textbook representations of molecules in this manner. This student also showed that s/he knew molecules had to move around within a cell to allow biochemical processes to occur which is in contrast to the student above who described biochemical processes as being structured with the molecules not moving around and colliding with each other:

I: Let's take some time to imagine we're sitting inside a cell right now and you can see everything inside that cell. Describe what you think the inside of the cell looks like

S: it look[s] [like] the river, like in the river where there are many microorganisms that are living in the river, so in the cell I can imagine a cell like eh like you are putting not really in the river; that you are taking a glass of water the the glass the outside part of the glass is the membrane and then the water will be the fluid in the cell then I put something inside the water to replace it as the nucleus and some other things so it can mix with the other molecules in the cell

I: how do you think the molecules forming a linear pathway are moving?

S: the molecular formula?

I: what do you think it looks like, if you were watching the molecules?

S: like balls, ah some of them may have irregular shapes some of them will be maybe squares

I: are they moving or staying still?

S: I think they are moving since the cytoplasm is fluid so they maybe it can happen that they are moving but definitely they need to be moving, because if they are just stationery which means the process the glycolytic pathway, or the pathways that are supposed to be happening in the cell will not happen because molecules need to move from one place to the other.

Although the above student described that molecules have to move from one place to another to allow processes to occur, this student did not convey whether s/he thought the

molecules were required to move around in order for them to collide, and if favourable, allow the reaction to occur. The above 2 quoted students are examples of where the use of abstract diagrams and a lack of content knowledge may hamper the formation of a useful mental model (Lowe, 1996).

After students had described what they thought the inside of the cell looked like, they were probed about how they thought biochemical processes “looked” in the cell, i.e. their mental models of processes occurring in cells. The following interview extract and accompanying student-generated diagram (Fig. 5.3) illustrates this student’s mental model of linear, cyclic and cascade biochemical processes:

I: we’ve just been inside the cell and you’ve described what you see in the cell, now come out of the cell and imagine we’re looking down a powerful microscope and you can still see all the molecules and everything else you’ve described inside the cell. Now I’m asking you to represent the molecules involved in a cyclic process by using letters, as if you were watching the process. [Student was then also asked to represent linear (red A→E) and cascade (red A→E outside cell) processes]

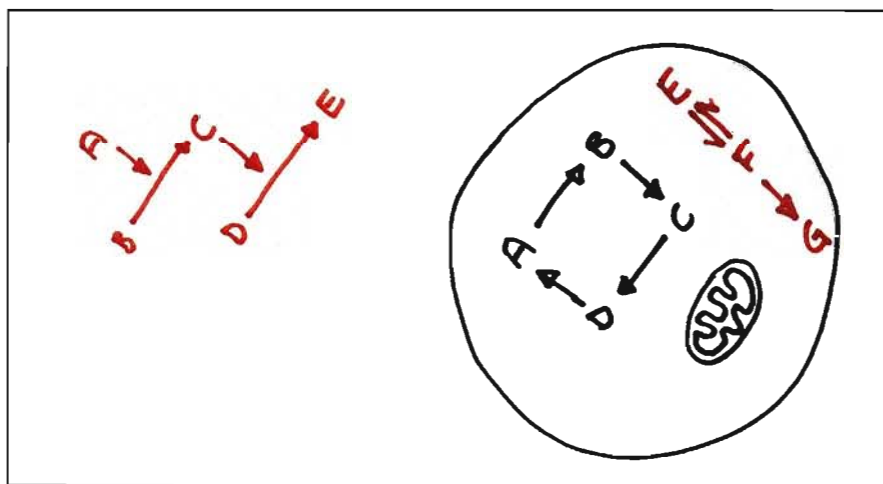


Figure 5.3 Student’s representation of biochemical processes *in vivo*

After the above student had generated Fig. 5.3 above, s/he was asked to describe how s/he thought the cell looked in terms of all the processes that were occurring as shown in the following interview extract:

I: ok, now can you describe the cell in terms of all those molecules, and processes [refers to student generated diagram in Fig. 5.3]

S: ok, describe each process?

I: yes you can, and I’d like to know what your cell’s made up of now

S: um... ok well... depending on what process, what process the cyclic process [refers to black cyclic process in Fig. 5.3] just um basically the compounds go in a circle and the end product D is needed to carry on the rest of the um, process, um, linear reactions [refers to

red linear process in cell outline in Fig. 5.3] E [points to "E"] um, you can have um going both ways from E to F or F to E, um, but basically linear reactions happen in a line ah, cascade reactions um... ah like the clotting process A [points to "A" of red cascade process in Fig. 5.3], A is like a factor that is required um to help change B to C and it works in a cascade like that [points to cascade process representation in Fig. 5.3].

The student quote above, serves as an example of how some students used LI to express their mental models of biochemical processes, both verbally and diagrammatically.

Furthermore, this next student also shows in a clear fashion that LI was often used to construct a mental model, namely, a linear process occurs in a line and a cyclic process in a circle inside the cell:

I: ok, let's say that we're sitting in the cytoplasm and we can see a cyclic process, for example the TCA, happening in front of us, describe what you think that will look like

S: aah, I think they would be going in a circle in front of me and you'll have products and various substances going off into the rest of the cell and ja, it would be going round and round

I: ok, and what about a linear process?

S: ok, also just a, ok it's not a circle it's just going straight and ja it's going to take all the carbohydrates and break them down [giggles] ok and whatever gets broken down and all those components are coming off and getting broken down in the cell

I: ok, let's come out of that cell and imagine we're looking at that same cell through a very powerful microscope, draw a rough outline of the cell

S: [draws cell outline in Fig. 5.4 below]

I: ok, now draw that cyclic process that you saw in the cytoplasm

S: it might look like.. [student is silent]

I: you can use letters like A, B, C, D to represent molecules

S: ok, I think it goes like that and [draws cyclic process in a circle in Fig. 5.4]...

I: ok, and what about a linear process?

S: [draws linear process "A" to "C" in Fig. 5.4]

I: ok, and how about a cascade?

S: um [draws cascade process "A" to "D" in Fig. 5.4]

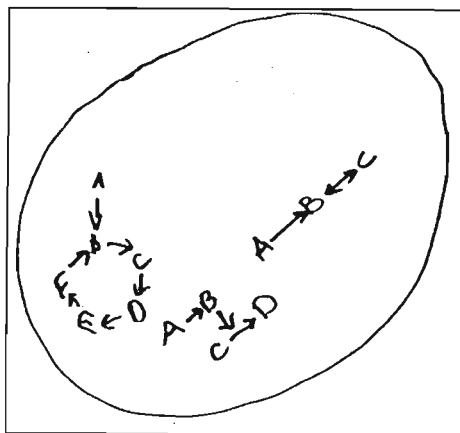


Figure 5.4: Student's representation of biochemical processes *in vivo*

Thus, the above 2 interview extracts and accompanying student-generated diagrams in Figs. 5.3 and 5.4 illustrate in both a diagrammatical (e.g. cyclic processes are drawn in circles) and verbal (e.g. the term “cyclic” used to describe a type of process) form, the rigid mental model of biochemical processes that students’ construct, based on the LI of conventional biochemical process representations.

In contrast to the above interview extracts, the following student, as a verbal output revealed his/ her mental model of cyclic biochemical processes as being dynamic systems whereby intermediates are regenerated. This same student was then asked to draw his/ her mental models of linear, cyclic and cascade processes *in vivo*, as illustrated in the following extract and Fig. 5.5 below:

I: Let's imagine we're sitting inside a cell right now and you can see everything inside that cell, even the molecules... imagine you could see a linear pathway happening, what do you think that would look like?

S: look like?

I: yes if you could see the molecules, what do you think a linear process would look like?

S: oh, ok, well you'll see one molecule and then something would happen to it and it will change to a different form and then something else would be added or taken away and it would change to another form, and it will carry on from one to another

I: and what about a cyclic process happening in the cytosol?

S: mm, I think then you'd see... um, because it doesn't end it's cyclic the intermediates are regenerated so they will be changed or whatever so they sort of get changed and then they go back to their original form so they can be reused in the next cycle, not like a linear where it has a clear beginning and end, it gets recycled and changed back

I: ok, so if you were watching a cyclic process describe what it would look like, how the molecules would be moving and reacting

S: I think for example you've got a compound A and it will change to compound B but as the cycle goes around or whatever they'll get changed back to their original form so they're then ready so when the next, say pyruvate or whatever comes through the cycle

I: ok, imagine we're looking at that same cell through a very powerful microscope and you can still see everything you saw when you were sitting inside the cell, can you draw a big outline of the cell for me

S: [draws cell outline in Fig. 5.5]

I: ok, now draw that linear process you saw, using letters to represent molecules

S: [draws linear process “A” to “D” in Fig. 5.5]

I: ok, what about what you think a cyclic process would look like?

S: [draws cyclic process in a circle in Fig. 5.5]

I: and a cascade?

S: can I use the same letters for the cascade?

I: yes that's fine

S: [draws cascade process “A” to “G” in Fig. 5.5]

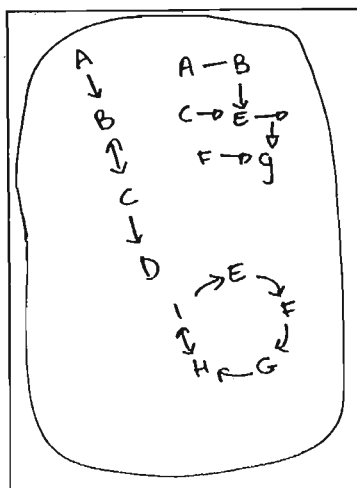


Figure 5.5: Student's representation of biochemical processes *in vivo*

The above quoted student was then asked to comment on the functioning of the cell (i.e. verbally reveal his/her mental model) in terms of the biochemical processes that s/he student had drawn in Fig. 5.5:

I: now that you've drawn all these processes that you saw inside the cell, describe how you think all these processes work, like describe how you think the cell looks with all these processes happening

S: ok, say now you've got this process happening, look at this one first [points to linear process in a straight line in Fig. 5.5], the say this comes from food that you've eaten and it gets broken down and you get A then it gets changed through a pathway to get D, then D ok, I haven't used the same letters but D might then feed into this cyclic pathway [points to cyclic process in a circle in Fig. 5.5] and then on the way little intermediates will be passed out, example ATP and NADH or whatever, then that might be used say ADP might be used in a parallel-coupled with another reaction and then something that came out of this reaction might go onto a cascade reaction.

Although the above student drew biochemical processes in the same manner as is found in textbooks i.e. with LI (see Fig. 5.5), this student correctly described biochemical processes as the changing of one intermediate into another and further described the integration of metabolic processes and sharing of intermediates. Thus, these students may not actually think these processes occur in these conventional structures *in vivo*. In fact the results suggest that these students hold mixed mental models of how the processes would look functioning *in vivo*. On the one hand, they think the processes in cells would look like the diagrammatic representations (i.e. they take them literally) but at the same time they are aware that the reactions in cells would not involve fixed structures but a dynamic movement of molecules so that a reaction can occur.

In the second part of these interviews, an alternative approach was used to gain further insight into students' mental models of biochemical processes. These involved questioning students about the conventional manner in which processes are depicted in textbooks by asking them to draw how they would represent linear, cyclic and cascade processes if they were textbook artists. We found that *all* 15 of the students who were interviewed drew the processes in the conventional textbook manner, even though they were given the freedom to draw these representations as they wished. Some of these students, however, knew that these processes did not occur in these structures. The following interview extract illustrates how this student is an example of a student who manifested the LI difficulty as s/he thought the biochemical processes occurred in conventional spatial arrangements within the cell:

I: ok, let's imagine that you're an artist for a text book and they'd like you to represent a cyclic process, you can use letters you don't have to remember names, how would you represent a cyclic process?

S: ...um I don't know I'm not very clear on this just basically... [draws round cyclic process in Fig. 5.6]

I: ok, and what about a linear process?

S: [draws linear process in a straight line in Fig. 5.6]

I: ok, and how about a cascade process?

S; [draws cascade process in step-wise form in Fig. 5.6]

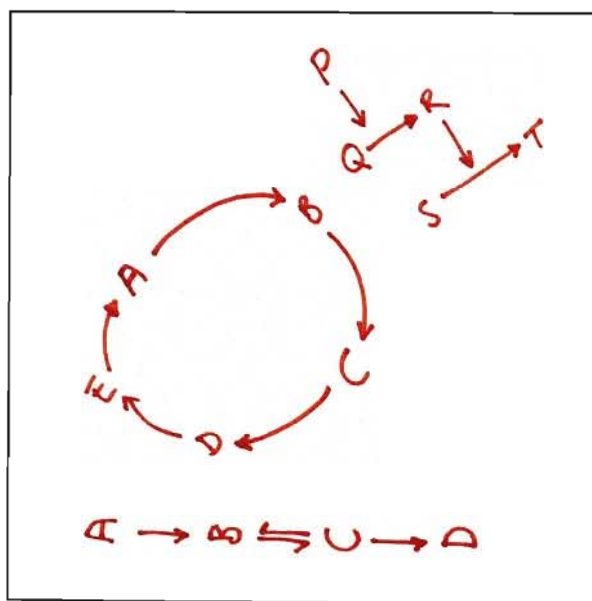


Figure 5.6: Student's textbook representation of biochemical processes

I: ok, tell me why you've chosen to represent a cyclic pathway in a circle [points to student's representation of a cyclic process in a circle in Fig. 5.6], if you're drawing for other students in a textbook?

S: um, I don't know maybe because that's the way I've always know it to be represented and because it makes sense to draw cyclic and a circle and I suppose you can draw it in a square as well is suppose

I: ok, so do you think it's a good idea to draw all cyclic pathway in a circle?

S: um ja well if I see a circle then I think it's a cyclic pathway I just associate it with that, but I don't know

I: and what about your other representations like your linear and your cascade [points to these two student representations in Fig. 5.6 respectively], can you think of any advantages or disadvantages of drawing them like that?

S: ja I can um, linear you can just see it's in a straight line and it's fairly easy to understand and ja even the cascade, that would be the way I would represent it ... and ja I understand it like that as well I know some students battle but that makes the most sense to me, ja

I: so now why do you think this has been called a cyclic process [refers to students diagram of a cyclic process in Fig. 5.6]

S: mm... well, it happens in a circle because they ok the substrate leads to that and that but then this end product E [points to "E" in cyclic process in Fig. 5.6] is used in then in the reaction for, to create A [points to "A" in cyclic process in Fig. 5.6] so it sort of carries on in a circle

I: so does it function in a circle?

S: functionally, ja

I: and what about linear pathways?

S: um... ja I would also say that functionally it happens in a line, A leads onto B [points to "A" and "B" in linear process in Fig. 5.6 respectively], you know there's no sort of leading back to A like there would be in a cyclic pathway

I: do you have any other comments about the structures of pathways?

S: ... mmm, no not really.

The above student illustrates that s/he has become so used to seeing cyclic processes in a circle and linear processes in a straight line that s/he associates these processes with their designated shapes. This is despite the fact that the interviewer attempts to get the student to link his/her mental model to the functioning of the processes. The above student also conveyed that s/he favoured the conventional representations of biochemical processes and s/he showed the LI difficulty. In a study by Dwyer (1969), investigating whether the diagrams 86 university students thought were effective corresponded to their achievement measured by 5 criterion tests, it was found that the diagrams students' thought were effective were not necessarily those that were effective in terms of student achievement, which is also illustrated by the above student's interview extract.

In contrast to the above student, although this next student favours the textbook representation of biochemical processes in the conventional manner, s/he realises that they do not occur like this in the cell. Thus, this student is an example of how some students would probably hold mental models that better reflect the functional and structural characteristics of biochemical processes within cells, as opposed to students who show LI.

The student quoted below was also questioned on his/her opinion of the names linear, cyclic and cascade for describing these types of biochemical processes as illustrated by the following interview extract:

I: Do you think linear, cyclic and cascade are good names to describe these processes then [refers to student's diagram showing conventional textbook representations of linear, cyclic and cascade biochemical processes]?

S: yes those names describe what is happening... a cascade is one reaction stimulates other reactions um, cyclic, circular um products reformed, um linear straight line it's not a circle only one product is formed...

I: ok, and now from what you've just said, do you think these processes have been named because of what they actually look like, or because of what they're doing?

S: what they're doing?

I: could it be that it's called a cyclic pathway because first of all it looks like a circle when it's occurring and because the products are reformed, or is it one or the other?

S: I think it's one or the other, I think that it's named cyclic because the product is then reformed, linear because it it you, the way you draw it is in order to understand easier it doesn't mean it happens like you draw it, if you know what I mean. So I think they're named by the processes that occur and not by the way you draw them, although it helps, it helps to draw it like cyclic meaning circle so it does help to link the name

I: do you find it confusing though that cycles are always drawn in circles?

S: no I don't think so

I: so if you had to draw a diagram in a textbook, would you always draw a linear pathway in a straight line and a cyclic pathway in a circle

S: yes I would.

The student quoted above conveyed that s/he thought the conventional representations helped to link the names of the processes to the manner in which they function. Therefore, s/he favoured the use of the terms "linear", for example, to describe the function of biochemical processes. This student therefore did not show the LI difficulty but may very well use the LI feature to classify processes based on the manner in which they are conventionally represented in textbooks, which in some cases may lead to an incorrect classification of a process (e.g. to probes in Fig. 5.2). By linking his/ her content knowledge about the functioning of biochemical processes, to the manner in which biochemical processes are depicted both verbally (e.g. by terms e.g. "cyclic") and diagrammatically (e.g. cyclic processes drawn in circle), the above student illustrates how an integrated mental model can be formed through visualising the processes by using the representation, as a form of cue to determine the function of a process. This is what textbook authors/ artists intend.

Thus, the above interview extract is an example illustrating that although some students use conventional representations to depict biochemical processes, they think more deeply

when it comes to visualising these processes within the cell and realise that the textbook depictions are mere representations of the biochemical processes. These students who may have used LI in response to their written probes (Sections 5.3.1 & 5.3.2) may, therefore, use the LI feature to identify processes but they do realise that they occur randomly in the cell. In relation to this, Reiss and Tunnicliffe (2001) stated that there is believed to be a difference in an individuals' mental and expressed model, which is why an individual should be asked to elicit more than one of their expressed models (as outlined in Chapter 3, Fig. 3.1). In this regard, our results show that the verbal data gathered from interviews with students is often closer to scientifically accepted biochemical process functioning than the diagrams generated by the students. However, a problem might arise as we saw in earlier interview extracts, whereby some students may superficially use LI to classify processes, and not look closely at the arrows and elements within the diagram that would determine what type of process it is. This finding supports the use of interviews to probe deeper into whether students use LI as a classification feature, or whether LI is a difficulty in their visualisation of biochemical processes, as analysis of students' written responses does not provide the required insight into students' LI difficulties.

5.4 SUMMARY AND CONCLUSIONS

Two major research questions were addressed in this chapter, namely what are students' mental models of biochemical processes, and secondly, do textbook representations of biochemical processes influence students' visualisation of these processes and thus the mental models students construct?

In terms of students' visualisation and thus mental models of biochemical processes, the student responses to various written and interview probes in this chapter revealed that there are scientifically acceptable, erroneous and mixed mental models of biochemical processes held by students in the sample. This illustrates a point we made earlier in Chapter 3 (Section 3.3, Fig. 3.1) that differences in students' expressed representations of their internal models may be possible. Although a diagram in many ways reflects the author's mental model of the depicted content (Lowe, 2001, pers. comm.), the student would need to interpret the diagram in the manner in which the author intended in order to understand

and build an effective mental model of the depicted content. The conventional representations of biochemical processes, therefore, reflect how experts' functionally understand a biochemical process. In cases where students literally interpret this conventional representation, students are taking the conventional representations of biochemical processes literally and are not interpreting the diagram in the manner intended by the author.

The conventional representation of biochemical processes (as they are seen in textbooks) by the students in interviews is consistent with work done by Kindfield (1993/1994 & 1992). She found that students literally drew chromosome representations as they were seen under a light microscope and the features they drew had no functional effect on the process of meiosis, when this was what they were asked to draw (Kindfield, 1993/1994 & 1992). In this regard, the students in our sample exposing LI may not have been visualising how the processes occur in the cell as they were still using their perceptual reasoning from the conventional textbook representations of the processes. It is important to note however, that in some cases even when students literally drew biochemical processes, some of these students verbally expressed that they did not visualise them to occur in this manner within the cell. This is in support of a claim made by Reiss and Tunnicliffe (2001), that there is believed to be a difference in an individual's mental and expressed model (see Chapter 3, Fig. 3.1). The presence of mixed mental models was also exposed in this chapter as some students were seen to use more than one feature of a process to classify a process diagram as being of a particular process type. For example, (in Section 5.3.2.2), a student who initially classified diagram 8 (in Fig. 5.2) as a cyclic process because it was drawn in a circle, noticed that there was no arrow between "E" and "F" and therefore changed his/her mind and classified process 8 as a linear process. This was because the student's feature that the starting and end metabolite of a cyclic process are the same, was not satisfied in diagram 8 (see Fig. 5.2).

Students with correct or mixed mental models of biochemical processes may favour the conventional manner of depicting processes, as it is the most appropriate in terms of creativity for the students. These students can, therefore, link the name and conventional depiction with the function of these processes. Some student's mental models that did not expose LI, even when they drew biochemical processes in the conventional manner,

illustrated this. These students may, therefore, be using a deeper level of processing whereby they have a higher level of content knowledge, recognise the conventions used to depict these processes and are able to decode the visual cues determining the relationships between the elements of the diagram (Lowe, 1991). By linking content knowledge about the functioning of biochemical processes to the manner in which biochemical processes are depicted both verbally (e.g. by terms e.g. “cyclic”) and diagrammatically (e.g. cyclic processes drawn in a circle), these students illustrate how an integrated mental model can be formed through visualising the processes by using the representation as a form of cue to determine the function of a process. In this respect, the importance of visual literacy and content knowledge in appropriate mental model construction was discussed in Chapter 2 (Sections 2.3.1 & 2.3.3.3 respectively).

In contrast, the students who exposed their incorrect mental models of biochemical processes, used LI as the 1st cognitive characteristic to classify processes. In many of these cases, students did not look closely at the elements in the diagram (i.e. the arrows and letters representing molecules) when they classified processes, thereby making the LI feature an overriding feature when these students classified diagrams depicting biochemical processes. In both the verbal and diagrammatic descriptions of their mental models of biochemical processes, students’ use of LI would constitute a surface-level reasoning difficulty. These students seem to be so familiar with the conventional method of depicting biochemical processes as seen in textbooks and therefore think or visualise that these processes look or occur in these structures in the cell. Glenberg and Langston (1992) support the use of diagrams as the spatial arrangement of the diagram elements allow them to be integrated into the mental model in the same way. This is in contrast to text where the sequential nature of the text requires more cognitive processing to accurately represent the information, especially where two steps are occurring at the same time for example (Glenberg & Langston, 1992). In the current study however, students were literally interpreting the manner in which biochemical processes are conventionally represented and were building these representations into their mental models of how these processes occur in the cell. These students may not be aware of the broad convention used in flow diagrams, where the arrangement of the diagram elements represents their connection and the sequence of events rather than the natural state of the system (Lowe, 2001, pers. comm.).

The remediation of the LI difficulty may be possible if students are exposed to multiple representations and the nature of the different biochemical processes were explained in both textbooks and lectures. Some students (data not shown in this chapter) did point out, when questioned on the conventional textbook representations that they drew of linear, cyclic and cascade processes, that they would like textbooks to include more information on their diagrams. For example, to either illustrate/ describe the integration of various biochemical processes or explain why they have been called cyclic and cascade for example. This is supported by Bodner (1991, as cited by Garnett *et al.* (1995)) who stated that alternative conceptions arise from the over-simplification of concepts (i.e. drawing processes in the conventional structures in our case) and explanations of the set limits should be included so that students are not misled. Analogies have also been found to aid student's understanding (e.g. a factory picture analogy of the cell for high school students (Bean *et al.*, 1990)), although differences in terminology between various disciplines should be pointed out (Garnett *et al.*, 1995). This is applicable to our study, as analogies are drawn between the nature of a process (e.g. cyclic) and the manner in which it is depicted (e.g. in a circle). However, some students who show the LI difficulty take this analogy literally and think that these processes occur in these structures within the cell. Although well designed diagrams are an effective means of portraying information, the diagrams do not contain all the information required and a level of prior knowledge is required to allow effective use and hence understanding of a particular domain from a diagram (Cheng *et al.*, 2001). The fact that all 15 students represented linear, cyclic and cascade processes in the conventional manner when they were asked in the interviews to draw them (see Figs. 5.3 & 5.6 for examples), may indicate that students have not thought about how these processes occur in the cell. It follows that these students' may favour the textbook depictions as the names and therefore conventional representations enable them to link the processes to their functions, as indicated by some student's responses. Furthermore, some students' who have the LI difficulty think these processes occur in these structures within the cell.

The dual coding theory may be applied to our results (e.g. Mayer, 2003). Two information processing systems i.e. one that presents information visually and the other verbally, have referential connections between them in the working memory that allow the integration of these two processing systems, thus allowing an individual to construct a "runnable" mental model of a situation (Mayer & Anderson, 1992). Students' with the LI difficulty may not

be building referential connections between their two information processing systems. This may account for why they see biochemical processes in their conventional depiction forms but are not relating these depictions to their content knowledge or how these processes occur in the cell.

Cheng *et al.* (2001) suggest that in order to generate well-designed or effective diagrams, an understanding of how individuals integrate information from the diagram into their knowledge and understanding of the domain is required. From this study on LI, we can thus conclude that although the conventional manner of depicting certain biochemical processes aids in helping students to link a particular process to its name (e.g. linear, cyclic and cascade) and thus function, the lack of multiple representations and explanations on these diagrams leads some students into thinking these processes occur in these spatial arrangements in the cell. The implications of the LI difficulty are that some students would view the cell as a rigid structure composed of structures of processes, and not a fluid and dynamic environment composed of various molecules that partake in a variety of integrated biochemical processes, as required by the demands of the cell. This is based on their singular representation system of biochemical processes, i.e. the manner in which they are conventionally depicted. There is, therefore, a need to develop more than one representation system for these biochemical processes and to include explanations about the nature of these processes. In this regard, Bodner and Domin (2000) have found that successful problem solvers (in organic chemistry) are able to switch between representation systems, and their mental representations of a system contain elements of more than one representation system. Content knowledge of a particular domain provides the framework for interpreting the diagram and allows the individual to make the appropriate links between diagram elements and the wider context in which the diagram elements fall (Lowe, 1994), thereby enabling a student to visualise the process and build a scientifically acceptable mental model of it. Therefore, by providing students with multiple representations of these biochemical processes, explanations of their nature, and a well-integrated content knowledge linking metabolic processes, the outcome of which would hopefully be that students would have a sound and integrated understanding of metabolic processes. This would promote inferences to be made and successful problem solving to occur, all of which are essential to the understanding and practice of biochemistry.

Through the research design and inductive analysis of the data gathered in this chapter, the research questions restated at the beginning of this section were answered. As a result, the following major findings of this chapter are that:

1. Some students were found to have difficulty with the visualisation of the spatial arrangement of biochemical processes, largely influenced by the conventional textbook representations used to depict the biochemical process;
2. Students' with this LI (literal interpretation) difficulty visualised linear, cyclic and cascade biochemical processes to occur within the same spatial arrangements as conventionally depicted in textbooks (i.e. in "straight lines", "circles" and "step-wise" forms, respectively), within the cell itself;
3. Some students were found to have mixed mental models in that, although they diagrammatically represented biochemical processes in their conventional arrangements, they verbally expressed that they did not visualise them to occur within these spatial arrangements within the cell itself;
4. In terms of the role of textbook representations on students' visualisation and mental models of biochemical processes occurring *in vivo*, our results show that although many students favour the conventional spatial arrangements, these diagrams have a negative effect on some students' visualisation and the mental models that they construct. Therefore, we suggest that multiple representations and explanations be included when instructing students on biochemical processes.

Chapter 6 and 7 that follow, investigate students' visualisation of the chemistry of individual biochemical reactions, using 2 examples of textbook diagrams depicting biochemical processes.

CHAPTER 6

Students' Use of a Textbook Diagram to Visualise the Chemistry of Two Glycolytic Reactions

6.1 INTRODUCTION

Glycolysis is an anaerobic oxidative process whereby 1 molecule of glucose is oxidised to form 2 molecules of pyruvate yielding a net gain of 2 molecules of ATP and 2 molecules of $\text{NADH} + \text{H}^+$ (Lehninger, 2000). A wide range of literature can be found on teaching and learning strategies to aid students' understanding of metabolism (e.g. Punekar, 2000 & Torres, 1993), with some of these studies focussed on the content of glycolysis (e.g. Schultz, 1997 & Ryder & Leach, 1996). Glycolysis as a context has also been investigated by Anderson and co-workers (e.g. Anderson *et al.*, 2002, pers. comm., Grayson *et al.*, 2001, Anderson *et al.*, 2000 & Anderson & Grayson, 1994). Anderson and Grayson (1994) realised that students had difficulty in understanding metabolism as a subject, which led them to develop an alternative teaching strategy that focussed on teaching students how to understand the functioning of metabolic pathways and aided them in acquiring skills for studying them, thereby eliminating the need for rote learning of the pathways by the students. Anderson *et al.* (2002, pers. comm. & 2000) have also identified various student conceptual and reasoning difficulties with the understanding of the glycolytic pathway, using the framework of Grayson *et al.* (2001), that can be extended and applied to student understanding of other metabolic pathways. Examples include the localised reasoning difficulty in which students consider the local and not global effects of inhibiting one of the enzyme catalysed reactions in a metabolic process, and the essential nature difficulties whereby students' do not recognise that ATP and the enzyme are essential intermediates in a reaction mechanism (Anderson *et al.*, 2002, pers. comm. & 2000, Anderson & Grayson, 1994). A concise description of these published difficulties by Anderson and co-workers is available on the conceptual and reasoning difficulties website at www.card.unp.ac.za.

Various studies in the area of chemistry (e.g. Johnstone, 1991) have reported students' difficulties with their visualization of chemical reactions. To our knowledge, no such studies have specifically focussed on student difficulties with the visualization of biochemical reactions, let alone with the use of visualization tools such as diagrams. Furthermore, a survey of the literature by Cheng *et al.* (2001) revealed that general claims about the effectiveness of diagrams were largely based on intuition rather than rigorous research. The aim of the study reported in this chapter was, therefore, to investigate student visualisation and understanding of biochemical reactions when using a diagram as a visualization tool. To achieve this, we selected two consecutive glycolytic reactions namely, the split reaction catalysed by fructose-1,6-bisphosphate aldolase (aldolase) and the isomerization reaction catalysed by triose phosphate isomerase, and employed the method of Grayson *et al.* (2001) to identify and classify any student difficulties (Chapter 3, Section 3.3.2). These difficulties were then considered in light of a model proposed by Schönborn *et al.* (2003 & 2002) that investigates the factors that affect student interpretation of diagrams. The nature and possible sources of these student difficulties was also considered with respect to various literature on the use of flow diagrams for teaching and learning. Furthermore, possible teaching interventions and remediation strategies are also suggested.

Thus, the following research questions were addressed in this aspect of the study: (1) What are students' models of the biochemical reactions being studied?; (2) Do the models reveal student difficulties with the visualization of the chemistry of these reactions?; (3) Does the diagram influence this visualization and, if so, in what way?; (4) What are the other possible sources of the visualization difficulties?

6.2 METHODS

6.2.1 The Study Group

Ninety-six 2nd-year biochemistry students participated in this study, although numbers fluctuated depending on whether, or not, students attended their lectures and tutorials. The students were attending a module entitled, "Integration of Metabolism in Living Organisms", in which glycolysis was taught. All 96 students responded to various written

probes (see Section 6.2.4) and 15 volunteers were interviewed. All students had already attended biochemistry courses, in their first semester, covering the following topics: amino acids, proteins, carbohydrates and lipid structure, an introduction to enzymes, pH and buffers and the immune system. During the “Integration of Metabolism in Living Organisms” course, and prior to this investigation, the students were taught the chemistry of the glycolytic pathway and attended a tutorial that required them to trace the path of Carbon-14 through the pathway by using their knowledge of the chemistry.

6.2.2 Description of the Diagram and Relevant Biochemistry

Fig. 6.1, below, is the diagram that was supplied to students when investigating their ability to visualize the chemistry of the split and isomerization processes (Reactions 4 and 5 respectively). This diagram was also supplied to students as part of their course notes and was taken from Lehninger (2000), one of the prescribed textbooks for the second year biochemistry metabolism course. To facilitate reading of this chapter a larger copy of Fig. 6.1 may be found in Appendix C.

The structures of the various glycolytic intermediates are shown on the right hand side of the pathway, with descriptions of some of the reactions in blue and pink boxes on the left of the appropriate reactions. The cytosolic enzymes have been omitted from this diagram but were, instead, discussed in the text, which was not supplied to students when responding to written and interview probes. Students did, however, receive the diagram’s caption (see Fig. 6.1) during the investigation, which they could consult on general details pertaining to glycolysis. Since the focus of the study was on reactions 4 and 5, we will not discuss the chemistry of the other reactions of glycolysis, but focus on these two specific reactions only. In this regard, more details on reactions 4 and 5 (see Fig. 6.1) are given in Figs. 6.2- 6.4 in the form of propositional statements to the probes found in these figures. In this chapter, the following abbreviations will be used for the intermediates of reactions 4 and 5 (see Fig. 6.1): G3P (glyceraldehyde 3-phosphate) and DHAP (dihydroxyacetone phosphate).

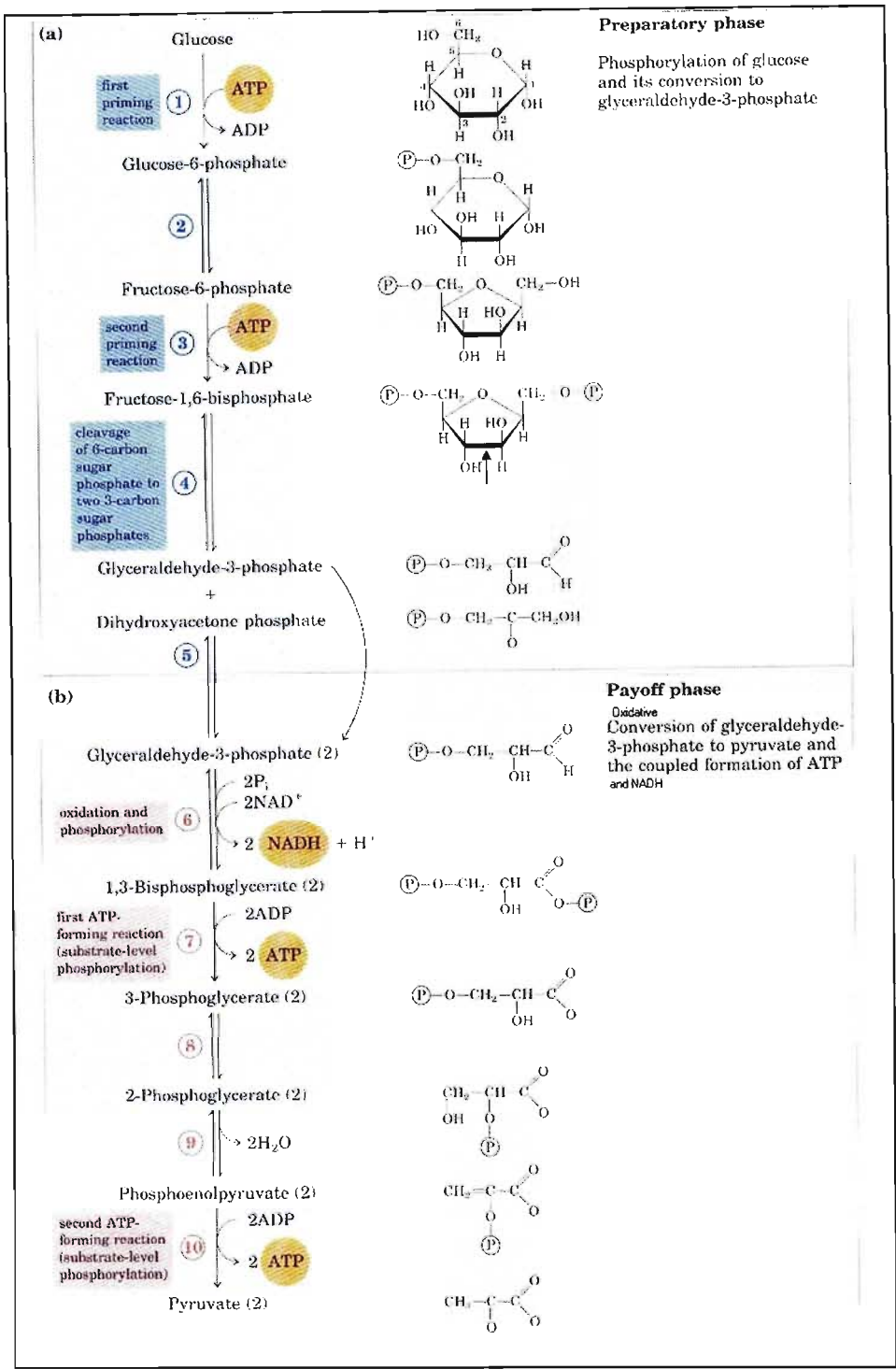


Figure 6.1: The two phases of glycolysis (Lehninger, 2000).

For each molecule of glucose that passes through the preparatory phase (a), two molecules of glyceraldehyde 3-phosphate are formed, both pass through the payoff phase (b). Pyruvate is the end product of the second phase of glycolysis. For each glucose molecule, two ATP are consumed in the preparatory phase and four ATP are produced in the payoff phase, giving a net yield of two ATP per molecule of glucose converted to pyruvate. The number beside each reaction step corresponds to its numbered heading in the text discussion. Keep in mind that each phosphoryl group, represented here as P , has two negative charges ($-\text{PO}_3^{2-}$).

6.2.3 Analysis and Classification of Student Responses

This is dealt with in detail in Chapter 3 (Section 3.3.7). Inductive analysis (McMillan & Schumacher, 1993) of student responses to various written probes (see Section 6.2.4), that were given to the students once every two weeks in tutorials, and interview probes, was used to identify various categories and sub-categories of students' conceptual, reasoning and visualization difficulties as presented in Section 6.3. These difficulties were then classified at levels 1, 2 or 3 on the research framework of Grayson *et al.* (2001). Section 6.3.4 considers the possible sources of student difficulties according to various literature on diagrams, and in light of a model proposed by Schönborn *et al.* (2003 & 2002) that investigated factors affecting student interaction with diagrams. Interviews were conducted according to the method of White and Gunstone (1992) (Chapter 3, Section 3.3.4). A video camera was used to accurately monitor where students were pointing on supplied diagrams, and the interview sessions were also audio taped and later transcribed for analysis. The video information was therefore used to supplement the transcribed audio information.

6.2.4 Written and Interview Probes: Design and Rationale

All written tests and interviews were conducted with the students post-instruction. A computer-scanned, coloured copy of Fig. 6.1 with the full figure legend accompanied each probe/ set of probes.

The difficulties investigated in this part of the chapter were initially classified as suspected (level 2, Grayson *et al.*, 2001), based on learner experience using the diagram in Fig. 6.1. Thus, these difficulties were further investigated with semi-focused type probes that were designed to obtain information about the difficulty without leading a student into a particular answer, a problem that can arise in the case of multiple-choice questions (Chapter 3, Section 3.3.3). The 2 semi-focused probes used in this study are presented in Figs. 6.2 and 6.3 below, together with the appropriate propositional statements. The rationale behind the design of the probes in Figs. 6.2 and 6.3 was to investigate students' visualization of the chemistry of reactions 4 and 5 and any related conceptual or reasoning difficulties. The probes in Fig. 6.2 required students to explain in words what they thought the chemistry of reaction 4 and 5 was, while the probe in Fig. 6.3 isolated reaction 4. Since Fig. 6.3 required students to write out the reaction, we wanted to see whether students

would consider Fig. 6.1 more carefully and thereby use the diagram as a visualisation tool to understand the chemistry of the reaction.

Use the diagram to explain in words what is happening in reactions (4) and (5).

Reaction 4.....

Reaction 5.....

Propositional statements:

Reaction 4: aldolase/ fructose-bisphosphate aldolase reaction

Fructose 1,6-bisphosphate \rightarrow Glyceraldehyde 3-phosphate + Dihydroxyacetone phosphate:

Fructose 1,6-bisphosphate, a 6-carbon sugar, is cleaved to form 2 3-carbon triose phosphates the structures of which can be seen in Fig. 6.1 on the right of the pathway (Lehninger, 2001 and Anderson, 2001, biochemistry course notes).

Reaction 5: Triosephosphate isomerase reaction

Dihydroxyacetone phosphate (DHAP) \rightarrow Glyceraldehyde 3-phosphate (reversible reaction):

DHAP and glyceraldehyde 3-phosphate (G3P) are isomers of each other (see structures in Fig. 6.1) and DHAP is converted to glyceraldehyde 3-phosphate, in a near equilibrium reaction in the cell, to carry on with the glycolytic pathway. Therefore 2 molecules of glyceraldehyde 3-phosphate are formed from 1 molecule of fructose 1,6-bisphosphate (Lehninger, 2001 & Anderson, 2001, biochemistry course notes).

Figure 6.2: Semi-focused type probe and propositional statements used to investigate student visualization of reaction 4 and 5 (Fig. 6.1)

Use the diagram provided to answer the following question(s) and support your answer(s) with an explanation.

Write a chemical equation that describes what is happening in reaction (4). For example in reaction (1): Glucose + ATP \rightarrow Glucose 6-phosphate + ADP.

Explanation....

Propositional statement:

Fructose 1,6-bisphosphate Glyceraldehyde 3-phosphate + DHAP

Figure 6.3: Semi-focused type probe and propositional statements used to investigate student visualisation of, and reasoning with reaction 4 (Fig. 6.1)

Following initial identification of difficulties, through analysis of student responses to the semi-focused type probes (Figs. 6.2 and 6.3), the two-tier multiple-choice type probe

(Treagust, 1988) in Fig. 6.4 was designed. In this manner, difficulties (see statements (a) and (c) Fig. 6.4), that had already been identified, were used as distractors. Incidence was also calculated and recorded. In these multiple-choice type probes, students were required to select an answer to a question and then motivate for their choice with an explanation (see Chapter 3, Section 3.3.3). By providing students with options (Fig. 6.4), we hoped to gain insight into how they *used* the diagram as a visualisation tool, i.e. whether they chose a particular option to the probe because it agreed with what they interpreted from Fig. 6.1.

Use the diagram provided to answer the following question(s) and support your answer(s) with an explanation.

!

Choose the statement(s) that describe(s) what happens in reaction (5):

(a). Glyceraldehyde 3-phosphate combines with Dihydroxyacetone phosphate to form Glyceraldehyde 3-phosphate (2)

(b). Dihydroxyacetone phosphate is converted to Glyceraldehyde 3-phosphate

(c). Dihydroxyacetone phosphate forms 2 Glyceraldehyde 3-phosphate

a b c (circle your selection(s))

Explanation of your choice(s).....

Propositional statements:

- The scientifically accepted answer is option (b)
- Dihydroxyacetone phosphate (DHAP) → Glyceraldehyde 3-phosphate (reversible reaction): DHAP and glyceraldehyde 3-phosphate (G3P) are isomers of each other (see structures in Fig. 6.1) and DHAP is converted by aldolase to glyceraldehyde 3-phosphate, in a near equilibrium reaction in the cell, to carry on with the glycolytic pathway. Therefore 2 molecules of glyceraldehyde 3-phosphate are formed from 1 molecule of fructose 1,6-bisphosphate (Lehninger, 2000 and Anderson, 2001 biochemistry course notes).

Figure 6.4: Two-tier multiple-choice type probe (Treagust, 1988) given to the students and propositional statements

Interviews with 15 student volunteers were used to gain greater insight into the nature and possible source of the visualization difficulties that had already been revealed in the written probes (Figs. 6.2- 6.4), and this process is described in detail in Chapter 3 (Section 3.3.4). The interviews enabled the classification of the difficulties at level 3 or partially established on the framework of Grayson *et al.* (2001).

Analysis of students' written responses to the probes in Figs. 6.3 and 6.4, as well as their interview extracts, revealed that some students had interactions between their difficulties, i.e. their interpretation of one part of the diagram representing a reaction (Fig. 6.1) was influencing their interpretation of another part of the diagram. Students' written responses were therefore once again analysed, compared and contrasted and the results are presented in Section 6.3.3.

6.3 RESULTS AND DISCUSSION

The results revealed evidence for students' sound visualization of, and visualization difficulties with, the chemistry of reactions 4 and 5 when using the glycolysis diagram (Fig. 6.1) as a visual aid. These difficulties are summarised in Table 6.1, below. The difficulties were collectively called Split Reaction (SR) and Isomer Reaction (IR) difficulties as these are the reaction mechanisms that occur in reaction 4 and 5 (see Fig. 6.1). In reaction 4, fructose 1,6-bisphosphate is cleaved or split to form one molecule each of both G3P (glyceraldehyde 3-phosphate) and DHAP (dihydroxyacetone phosphate). Subsequently, in reaction 5, the DHAP molecule is enzymatically converted into its isomer G3P to yield a second molecule of this latter compound (see propositional statements in Figs. 6.2- 6.4). The SR and IR difficulties were each classified into two sub-classes i.e. SR1 and SR2, and IR1 and IR2 respectively. A summary of the classification and description of all these difficulties are presented in Table 6.1 below.

Table 6.1: Classification (Grayson *et al.*, 2001) and description of student difficulties with their visualization of reactions 4 and 5 (Fig. 6.1) of glycolysis

Difficulty Class			Difficulty Subclass	
Code	Name	Description	Code	Description and Classification
SR	Split Reaction difficulties	Students have difficulty with identifying the cleavage products of fructose 1,6-bisphosphate in reaction 4	SR1	Fructose 1,6-bisphosphate \rightarrow 1 G3P Students think that fructose 1,6-bisphosphate is cleaved to only form 1 molecule of G3P in reaction 4 (Level 2)
			SR2	Fructose 1,6-bisphosphate \rightarrow G3P(2) Students think that fructose 1,6-bisphosphate is cleaved to only form 2 molecules of G3P in reaction 4 (Level 2)
IR	Isomer Reaction difficulties	Students have difficulty with determining how the final 2 molecules of G3P (G3P(2)) are formed in reaction 5	IR1	G3P + DHAP \rightarrow G3P(2) Students think that both the DHAP and G3P molecules need to react with each other to form the 2 molecules of G3P in reaction 5 (Level 3)
			IR2	DHAP \rightarrow G3P(2) Students think that the DHAP molecule forms 2 molecules of G3P in reaction 5 (Level 2)

Based on past learning experience by the present author, it was anticipated that the students in this sample might have had problems with interpreting reactions 4 and 5 (see Fig. 6.1). However, the nature of the SR and IR difficulties (see Table 6.1) was unanticipated. As the SR and IR difficulties were not revealed in any free response questions given to the students in the sample, for example: “Describe everything this diagram is meant to represent or show”, they were investigated with semi-focused type probes (see Figs. 6.2 & 6.3) and a multiple-choice (see Fig. 6.3) type probe, as well as during interviews.

The SR and IR difficulty classes and their subclasses will now be dealt with under the appropriate sections below along with students’ correct responses to the various probes in Figs. 6.2- 6.4.

6.3.1 The Split Reaction (SR) Difficulties

The SR difficulties were subdivided into the SR1 and SR2 subclasses as described in Table 6.1. These will now be dealt with in Sections 6.3.1.1 and 6.3.1.2, respectively. Thereafter, we present students' correct answers/ conceptions (Section 6.3.1.3).

6.3.1.1 The SR1 Difficulty Subclass

The SR1 difficulty subclass (see Table 6.1), which emerged from student responses to the probe about reaction 4 in Fig.6.3, is illustrated by the following quotes:

1. "The fructose 1,6-biphosphate [bisphosphate] is converted to Glyceraldehyde 3-phosphate."
2. "Fructose 1,5 [1,6] bisphosphate is being converted into Glyceraldehyde-3-phosphate. The structure is being changed from a six ring structure to a three ringed. This is also reversible so glyceraldehyde 3-phosphate is being changed to fructose-1, 5 [1,6]-biphosphate [bisphosphate]."

Quotes 1 and 2 both indicate that these students thought that fructose 1,6-bisphosphate forms 1 molecule of G3P, i.e. a 6-carbon compound forms one 3-carbon compound. Student 2 manifests this possible visualisation difficulty whereby s/he visualises the changing of a 6 to a 3-ringed structure. The above quotes that illustrate the SR1 difficulty are represented diagrammatically, by the following student generated diagrams in response to the probe in Fig. 6.3:

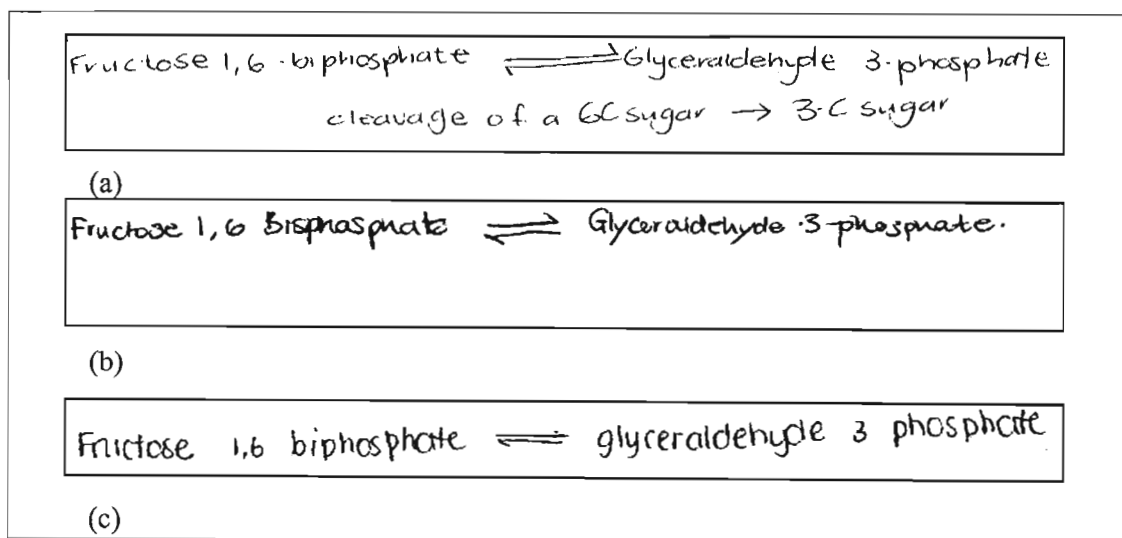


Figure 6.5 (a)– (c): Student generated diagrams erroneously illustrating the cleavage of fructose 1,6-bisphosphate (reaction 4, see Fig. 6.1) in the glycolytic pathway

The students quoted above indicated that they thought only 1 molecule of G3P was formed from the cleavage of fructose 1,6-bisphosphate. This could be due to difficulties associated with the visualisation of the chemistry of the split reaction (reaction 6, Fig. 6.1). Alternatively, this could be attributed to a lack of understanding of the meaning of split and/or cleavage reactions, difficulties reasoning with the diagram, or simply a confusing diagram. Furthermore, students may simply not have read the information provided in the blue box next to reaction 4 (see Fig. 6.1), and hence, may not have seen that there was no “2” written next to the first G3P molecule in the glycolytic pathway and, therefore, answered that only 1G3P is formed from fructose 1,6-bisphosphate cleavage in reaction 4 (see Fig. 6.1). In this regard, DHAP may possibly have been seen by these students to be an additional intermediate added into the glycolytic pathway at this point (reaction 4, Fig. 6.1). A reason for this might be, for example, the “+” sign between G3P and DHAP in Fig. 6.1, which is discussed in more detail in Section 6.3.4 where possible sources of the SR and IR difficulties are also considered.

The incidence of the SR1 difficulty was calculated as 16.3% (13/80) from student generated diagrams in response to the probe in Fig. 6.3. An interview extract illustrating the SR1 difficulty subclass is dealt with in Section 6.3.3 where the interactions between the SR and IR difficulty classes are also discussed. In Section 6.3.4 we suggest possible sources of this difficulty based on the model proposed by Schönborn *et al.* (2003 & 2002). The SR1 difficulty was classified at level 2 on the framework (Grayson *et al.*, 2001) as further interviews are required to gain insight into the nature of this difficulty to classify it at level 3.

6.3.1.2 The SR2 Difficulty Subclass

A description of the SR2 difficulty subclass is provided in Table 6.1. The following quotes, about reaction 4, in response to the probe in Fig. 6.2, show how students may be aware that 2 molecules of G3P are ultimately formed from 1 molecule of fructose 1,6-bisphosphate (reaction 5, see Fig. 6.1). This may be due to their content knowledge, but they may not see DHAP as being one of the products of the intermediate reaction (reaction 4, see Fig. 6.1):

1. “Fructose 1,6 bisphosphate is cleaved to form 2 molecules of glyceraldehyde 3 phosphate. This is easily done as the fructose molecule has been twice phosphorylated, therefore producing 2 equal molecules of glyceraldehyde 3 phosphate.”
2. “Fructose 1,6-bisphosphate gets cleaved into two Glyceraldehyde 3-phosphate.”

3. "This is a reverse [reversible] reaction where the 2 3carbon sugars can become fructose 1,6 bisphosphate or 2 molecules of glyceraldehyde 3 phosphate."

Quotes 1-3 above, are scientifically acceptable and provide a summary of reaction 4 and 5. However, the mechanism of reaction 4 was being probed and, therefore, by asking students to write out reaction 4 (Fig. 6.1), we were curious as to whether students might consider the diagram (Fig. 6.1) more carefully and correctly interpret reaction 4's mechanism. However, the following student generated diagrams in response to Fig. 6.3 about reaction 4 reiterate the SR2 difficulty as shown in Fig. 6.6 (a) –(c) below.

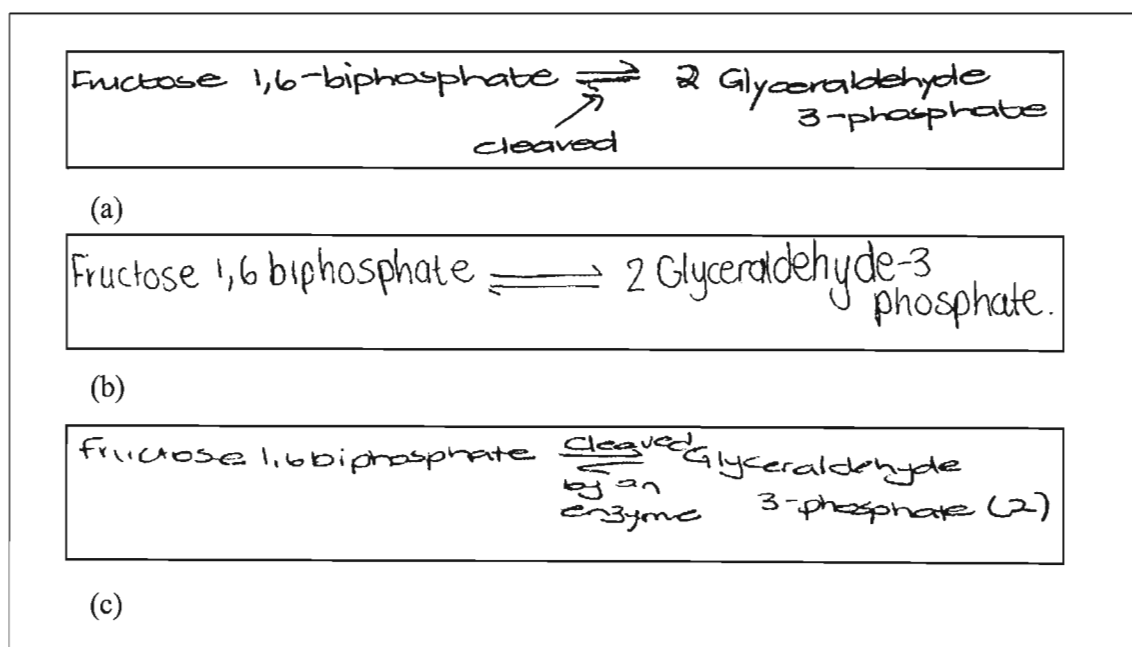


Figure 6.6 (a)– (c): Student generated diagrams erroneously illustrating the cleavage of fructose 1,6-bisphosphate (reaction 4, see Fig. 6.1) in the glycolytic pathway

The student responses above (Fig. 6.6 (a)-(c)) are correct in that 2 molecules of G3P are ultimately formed from fructose 1,6-bisphosphate, but in response to the probe in Fig. 6.3, they are incorrect. This is because the intermediate step, whereby 1 molecule of DHAP and 1 molecule of G3P are formed, has been omitted by these students and is the step that is depicted by reaction 4 in the diagram of glycolysis (Fig. 6.1). The above students, therefore, seem to correctly visualise that cleavage is occurring. However, they may not be visualising the structures of the intermediates and reaction mechanism itself.

The students showing the SR2 difficulty might have done so for a number of reasons, as discussed in more detail in Section 6.3.4. Firstly, in terms of the students themselves, they may not have had the required amount of content knowledge about the reactions mechanisms or an effective mental model to visualise the cleavage mechanism of reaction 4 (Fig. 6.1). This important role of content knowledge in diagram interpretation and mental model construction (e.g. Lowe, 1999) has been discussed in detail in Chapter 2 (Section 2.3.3.2 & 2.3.3.3). Secondly, by using the diagram as a visualisation tool, students with the SR2 difficulty may have used the wording in the blue box next to reaction 4 (see Fig. 6.1) and their content knowledge that 2 molecules of G3P are ultimately formed, to interpret or diagrammatically reason about the reaction mechanism. Furthermore, these students might have seen DHAP as being an additional intermediate added into the pathway, as interpreted by the “+” sign between DHAP and G3P (see Fig. 6.1, reaction 4), and if they had not considered the stoichiometry i.e. that there is no “2” written near the first G3P of the glycolytic pathway, they would be using the information provided in the blue box (i.e. that “two 3-carbon sugar phosphates” are formed) in their interpretation. Alternatively, the curved arrow between G3P and G3P(2) (Fig. 6.1) may have been interpreted as part of reaction 4, as there is no number next to the arrow as in the other reactions. This may have led students to think that fructose 1,6-bisphosphate is cleaved into 2 molecules of G3P, and that DHAP is an additional molecule that is added into the glycolytic pathway to form another molecule or 2 molecules of G3P. Thus, although students with the SR2 difficulty incorrectly thought that the end product of reaction 4 (see Fig. 6.1) was 2 molecules of G3P, and not 1 molecule of G3P and 1 molecule of DHAP, we were unsure how they visualised the reaction mechanism of reaction 4 (Fig. 6.1).

The incidence of the SR2 difficulty was calculated as 12.5% (10/80) from the student generated diagrams in response to the probe in Fig. 6.3. It was thus classified at level 2 on the framework (Grayson *et al.*, 2001). Subsequently, during interviews none of the students in our interview sample showed evidence of the SR2 difficulty subclass.

The SR1 and SR2 difficulties (Sections 6.3.1.1 and 6.3.1.2 respectively) indicate that in total approximately 28.8% (23/80) of students have difficulty with identifying the products of fructose 1,6-bisphosphate cleavage in reaction 4 of Fig. 6.1. In this regard, Section 6.3.3

presents data illustrating how students may have a combination of SR1, SR2 and correct conceptions (as dealt with in the following section), with correct or incorrect understandings of the subsequent reaction 5 (Fig. 6.1).

6.3.1.3 Students' Correct Visualisation of the Split Reaction

In order to ensure that a question is valid and fair (discussed in detail in Chapter 3, Methods, Section 3.3.5) it is essential that some of the students in the sample were able to answer the questions correctly. This was evaluated by comparing their answers to the propositional knowledge statements provided with each probe in Fig. 6.2- 6.5. Thus, by providing examples of students' correct answers, not only is the validity of the probes illustrated, but also these student responses provide examples of students' sound or correct visualisations of the reaction mechanism of reaction 4 (Fig. 6.1). The following 2 quotes are examples of students' correct responses to the written probe in Fig. 6.2 about reaction 4:

1. "Reaction 4: Fructose 1,6 bisphosphate is a 6C [6-carbon] ring. It is cleaved into two 3-C chains (Glyceraldehyde 3-phosphate and Dihydroxyacetone phosphate), each with a phosphate group attached to them. This is a reversible reaction that is not coupled in parallel with ATP. This is during the preparatory [preparatory] phase."
2. "In reaction 4- The fructose 1,6-bisphosphate ring is being cleaved in a reversible reaction to form Glyceraldehyde 3-phosphate and Dihydroxyacetone phosphate."

Quotes 1 and 2 above, illustrate how students' express their correct visualisations of the cleavage reaction mechanism depicted by reaction 4 in Fig. 6.1. In this regard, the following variety of student generated diagrams (Fig. 6.7 (a)- (e)) in response to the probe in Fig. 6.3, further illustrate students' scientifically acceptable conceptions.

Fifty-seven out of eighty (71.3%) students correctly answered the probe in Fig. 6.3, compared to 28.8% (30/80) who showed the SR difficulties. Thus a high percentage of students demonstrated sound visualization of the split reaction despite the somewhat confusing nature of the diagram. In addition, the high percentage of correct responses constitutes strong evidence for the validity of the probes employed i.e. the probes were probing what we intended them to probe. However, data showing how some of these 57 students also showed the IR1 and IR2 difficulty subclasses, with respect to what they thought happened in reaction 5 of Fig. 6.1, (see Table 6.1) will be discussed in Section 6.3.3. But first, we present the IR difficulties.

6.3.2 The Isomer Reaction (IR) Difficulties

The IR difficulties encompass difficulties students have with interpreting reaction 5 (see Fig. 6.1) and are made up of the IR1 and IR2 subclasses (Table 6.1) that are dealt with in Sections 6.3.2.1 and 6.3.2.2 respectively. Examples of students' scientifically sound visualisation, or correct answers to the probes in Figs. 6.2 and 6.4, are dealt with in Section 6.3.2.3.

6.3.2.1 The IR1 Difficulty Subclass

The emergence of the IR1 difficulties, whereby students think that the G3P and the DHAP molecules need to react with each other in some manner to form G3P(2) in reaction 5 of Fig. 6.1, (see Table 6.1) are shown below. This is in response to both semi-focused (Fig. 6.2) and two-tier multiple-choice (Fig. 6.4) type probes.

1. "Glyceraldehyde-3-phosphate combines with Dihydroxyacetone phosphate and is being changed into two glyceraldehyde-3-phosphate molecules which is also a reversible reaction." [Response to Fig. 6.2, about reaction 5]
2. "The glyceraldehyde 3-phosphate can continue alone or combine with dihydroxyacetone [phosphate] to form the 2 molcs [molecules] of Glyceraldehyde 3 phosphate. By the combination of DHA [dihydroxyacetone] phosphate and G3P [glyceraldehyde 3-phosphate] one obtains not 1 but 2 G3P molcs [molecules]." [Response to Fig. 6.2, about reaction 5]
3. "Glyceraldehyde 3 phosphate must combine with dihydroxyacetone phosphate to form Glyceraldehyde 3 phosphate (2) i.e. dihydroxyacetone canot [cannot] by itself convert to Glyceraldehyde 3 phosphate." [Response to option (a) in Fig. 6.4]
4. "Glyceraldehyde-3-phosphate + dihydroxyacetone phosphate \rightarrow glyceraldehyde-3-phosphate (2). 3 carbons + 3 carbons \rightarrow 3 carbons ($\times 2$) = 6 carbons. All reactions must be balanced." [Response to option (a) in Fig. 6.4]
5. "The two combine so that two of the glyceraldehyde 3-phosphate can form. There is also the addition sign which shows that they react/ combine with one another to form one product." [Response to option (a) in Fig. 6.4]

6. "There is a "+" sign, which stands for combines, it's a short hand in scientific notation."
[Response to option (a) in Fig. 6.4]

The above quotes indirectly illustrate the inductive nature of identifying and classifying students' difficulties (see Chapter 3, Sections 3.3.2 & 3.3.7). Once student difficulties have been identified and classified in response to free-response or semi-focused (e.g. quotes 1 and 2) type probes, they are honed into with the use of 2-tier multiple-choice type probes (e.g. quotes 3-6).

The above students showed the IR1 difficulty by stating that the G3P and the DHAP are both required to form another 2 molecules of G3P indicating, therefore, that these students may think that a total of 3 molecules of G3P are formed as a result of reactions 4 and 5 in Fig. 6.1. In this regard, quote number 2 suggests that the student may be reading the curved arrow between G3P and G3P(2) (see Fig. 6.1) as being the point where G3P can continue along the glycolytic pathway, or where it can combine with DHAP in reaction 5 to form another 2 molecules of G3P. Thus, as shown by du Plessis *et al.* (2002), the use of different types of arrow symbolism was shown to confuse students. In our case the straight reversible arrow indicates a reaction, but the curved arrow indicates the continuation of a molecule (see reaction 4 & 5, Fig. 6.1).

The 4th student quoted above, incorrectly reasoned about the reaction using stoichiometry to balance out the equation. This student would have been correct if s/he had realised that the DHAP is converted via an enzyme to G3P (without any interaction with the original G3P molecule) which results in the formation of the second G3P molecule. In addition, quotes 4- 6 illustrate the possible confusion surrounding the use of the addition sign ("+") on the diagram (see Fig. 6.1, reaction 5). These students stated that the "+" sign is indicative of the DHAP and the G3P molecules reacting with each other. Thus, the presence of "+" signs on both the reactant and product sides of reactions, may be another source of confusion for students when interpreting reaction 5 (Fig. 6.1).

At this stage of the investigation, there was evidence from students' written responses that there may be an interplay between the SR and IR difficulties in that what students' visualised happening in reaction 4, might influence their visualisation of reaction 5 (in Fig. 6.1), and vice versa. This interaction between the SR and IR difficulties is discussed in Section 6.3.3.

Interviews were conducted with students in order to gain greater insight into the possible sources and nature of the IR difficulties. In this regard, the IR1 difficulty that emerged in some student's written responses was found to be corrected by some students during interviews. By the time interviews were conducted (about 2 weeks after the written probes were given to the students), students had written a class test on the glycolytic pathway and would therefore have consulted their course notes issued by the lecturer, in which another representation of the glycolytic pathway (see Fig. 6.8) was presented. The following student was asked about this second diagram when s/he was found to have corrected her previous IR1 difficulties:

I: Have you seen any other diagrams of glycolysis?

S: there were various diagrams from pyruvate down to glucose [glucose down to pyruvate]

I: do they all look like this diagram?

S: no there's a change with the glyceraldehyde 3-phosphate it branches off um, and the glyceraldehyde 3-phosphate and the dihydroxyacetone phosphate are separate they're not just joined by a plus [+] and um, the dihydroxyacetone there's an arrow pointing towards the glyceraldehyde 3-phosphate saying it forms glyceraldehyde 3-phosphate, so that's how they get the two molecules because both of them form glyceraldehyde 3-phosphate

I: do you prefer that diagram to this one?

S: ja, it makes much more sense

I: so did that diagram help you with understanding what's happening in reaction 5, in this diagram [points to reaction 5 in Fig. 6.1]?

S: ja definitely.

The above student's response indicates that the addition sign ("+") at reaction 5 (see Fig. 6.1) was initially a source of confusion, and that s/he preferred the alternative diagram in his/her course notes (see representation of reactions 4 and 5 in Fig. 6.8), to Fig. 6.1. Through exposure to a different diagram, the above extract constitutes evidence for how conceptual change may occur. Conceptual change has been described as the replacement or integration of new concepts (Garnett *et al.*, 1995), leading to more integrated and cohesive knowledge in an individual's cognitive structure (Pearsall *et al.*, 1997).

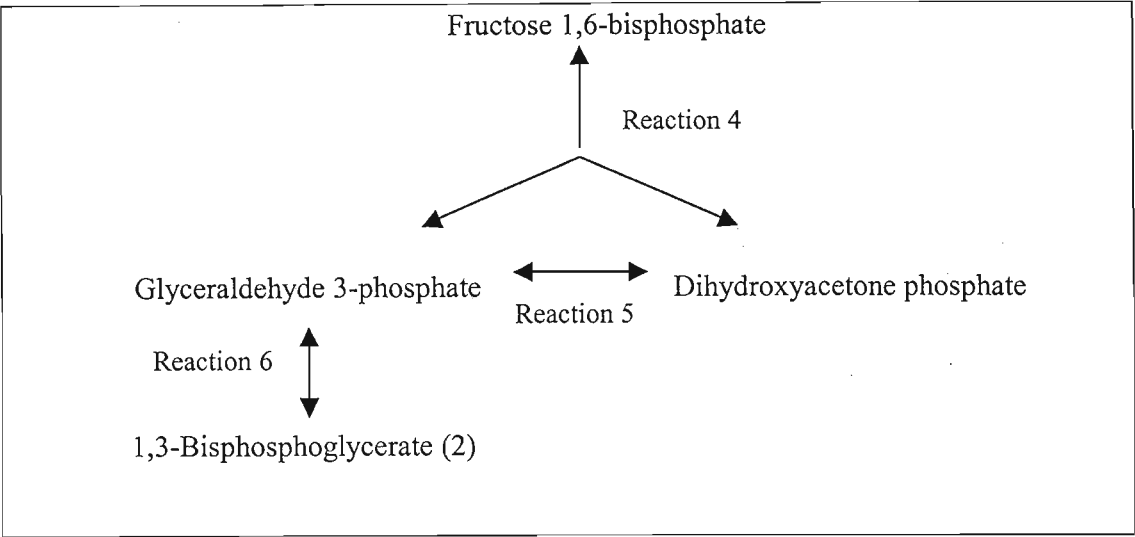


Figure 6.8: Alternative illustration depicting reactions 4, 5 and 6 (see Fig. 6.1) of the glycolytic pathway (adapted from Anderson, 2001 biochemistry course notes).

In Fig. 6.8, the “split” that occurs in reaction 4 is clearly depicted by the diverging arrow from fructose 1,6-bisphosphate, which replaces the addition sign found at reaction 5 in Fig. 6.1. Thus, the formation of the second molecule of G3P is also clearer to the student where another reversible arrow (reaction 5, Fig. 6.8), is used to show the conversion of the molecule of DHAP to a molecule of G3P. Therefore, the addition sign (“+” at reaction 5, Fig. 6.1) is not present in Fig. 6.8 to confuse the student. The disappearance of the IR1 difficulty by the above student, after exposure to another diagram, suggests that the diagram in Fig. 6.1 was possibly the major source of the IR1 difficulty, and probably stemmed from the inappropriate use of symbolism in the diagram. This issue is further discussed in Section 6.3.4 in relation to the model of Schönborn *et al.* (2003 & 2002) that investigates factors that affect students’ interpretation of diagrams.

The interview extract below also suggests that the diagram is a possible source of the IR1 difficulty. In this case, the student explicitly states that the area of confusion is the addition sign (+) and the arrow connecting G3P and G3P(2) (reaction 5, Fig. 6.1) and that the diagram is, therefore, unclear at this point. This particular student clearly conveys in his/her interview that the diagram seems to depict that the G3P and DHAP molecules are combined and then split to form the final 2 molecules of G3P, although s/he is aware that this does not seem feasible:

I: Is there any you find confusing about this diagram [points to Fig. 6.1]?

S: ... not, but ja this that um reaction of glycolysis ja where it's got the glyceraldehyde 3-phosphate plus the dihydroxyacetone phosphate [points to reaction 5 in Fig. 6.1], they don't tell you what they do with it, whether you're combining them and then resplitting them, it seems a but stupid, but that's what it seems to depict, or it's you're taking the glyceraldehyde 3-phosphate and then you're changing the dihydroxyacetone phosphate to make another glyceraldehyde 3-phosphate or what. But it seems like they're adding; it seems like they're bound together and then resplit

I: so what exactly about that section [points to reaction 5 in Fig. 6.1] of the diagram confuses you?

S: the plus [points to "+" sign at reaction 5 in Fig. 6.1]

I: the plus sign [points to "+" sign at reaction 5 of Fig. 6.1]?

S: yes

I: and what about this arrow going from glyceraldehyde 3-phosphate to glyceraldehyde 3-phosphate (2) [points to arrow between G3P and G3P(2)]?

S: well that shows you I think that, you get the two, I don't know one of the two is from the top one [points to glyceraldehyde 3-phosphate] I suppose, I don't know.

From analysis of the student responses to the various written probes (Figs. 6.2 & 6.4) and interview extracts, we were able to classify the IR1 difficulties at level 3 on the framework of Grayson *et al.* (2001). This is because the IR1 difficulty had been systematically investigated in a limited context. The 55.6% (50/90) incidence of the IR1 difficulty was calculated from students' choices and supporting explanations to option (a) of the multiple-choice type probe in Fig. 6.4. Interviews with students suggested that the IR1 difficulties may be diagram in nature as the addition sign ("+") at reaction 5 and the arrow between G3P and G3P(2) in Fig. 6.1, were a source of confusion for some of the students who showed these IR1 difficulties. However, some of these students were seen to correct their IR1 difficulties after exposure to a different diagram (Fig. 6.8) of glycolysis. Figure 6.8 excluded the "+" and arrow signs (seen in Fig. 6.1) and, in addition, students had subsequently learnt for a test which probably improved their content knowledge. This suggests that the IR1 difficulties may also be due to poor diagrammatic reasoning and conceptual knowledge. In Section 6.3.4 we apply our results to the model of Schönborn *et al.* (2003 & 2002) that focuses on factors, in order to move closer to the possible sources of the IR1 difficulties (Section 6.3.4).

6.3.2.2 The IR2 Difficulty Subclass

Reaction 5 in Fig. 6.1 was initially investigated with the use of a semi-focused probe (in Fig. 6.2) as it was anticipated that the students might have difficulty in interpreting the

manner in which these reactions are depicted, although the nature of the difficulties were unanticipated. Students who showed the IR2 subclass of difficulty (see Table 6.1) thought that the DHAP molecule formed the G3P(2) or two molecules of G3P, through various mechanisms. However, they do not seem to realise that G3P and DHAP are isomers of each other, as illustrated by the following examples of quotes:

1. "Dihydroxyacetone phosphate yields two molecules of Glyceraldehyde-3-phosphate from one molecule-replicates." [Response to Fig. 6.2, about reaction 5]
2. "The dihydroxyacetone phosphate is also being cleaved due to the repulsions between phosphate molecules within the compound to form glyceraldehyde 3-phosphate (2)." [Response to Fig. 6.2, about reaction 5]
3. "The dihydroxyacetone phosphate is spilt into 2 molecules by the loss of a H₂O molecule." [Response to option (c) in Fig. 6.4]
4. "Dihydroxyacetone phosphate splits into 2 molecules of Glyceraldehyde 3-phosphate due to the splitting and rearrangement of the six carbon sugars." [Response to option (c) in Fig. 6.4]
5. "Dihydroxyacetone phosphate is oxidized to form 2 molecules of glyceraldehyde 3-PO₄³⁻ [phosphate]." [Response to option (c) in Fig. 6.4]
6. "The dihydroxyacetone phosphate has the essential enzymes and energy to make an[other] 2 molecules of glyceraldehyde 3-phosphate." [Response to option (c) in Fig. 6.4]
7. "The dihydroxyacetone phosphate is an enzyme that provides the extra phosphates for the formation of 2 glyceraldehyde 3-phosphate molecules." [Response to option (c) in Fig. 6.4]
8. "Since altogether 2 glyceraldehyde 3-phosphate is passed thru [through] the payoff [phase], it would seem that dihydroxyacetone phosphate is converted to 2 glyceraldehyde 3-phosphate." [Response to option (c) in Fig. 6.4]

The above quotes suggest that none of these students considered the stoichiometry of the reaction in order to answer the written probes but, instead, came up with a variety of chemical mechanisms whereby DHAP forms the G3P(2). For example, student 1 seems to think that DHAP has the ability to "replicate" to form 2 molecules of G3P, perhaps by linking to his/her DNA replication content knowledge, although s/he does not indicate where he/she thinks the additional atoms come from to form this extra molecule. In another example, student 2 thinks that the 1 molecule of DHAP is cleaved to form 2 molecules of G3P and, as with student 1, may not have considered the structures of the individual molecules drawn in at the reactions (see Fig. 6.1). These two students, who first exposed the IR2 difficulties, were not present in the lecture in which the probe in Fig. 6.4 was given to the students. Further examples of the reaction mechanisms whereby some students with the IR2 difficulty thought 1 molecules of DHAP forms 2 molecules of G3P is given by students 3- 8. In this regard, student 3 thinks that a water (H₂O) molecule is removed from the DHAP resulting in the formation of 2G3P molecules, student 4 that the DHAP is a 6-carbon sugar that is cleaved to form 2 molecules of G3P and student 5 that

DHAP is oxidised which is the loss of electrons or hydrogen atoms. Furthermore, student 6 seems to envisage the DHAP as having energy and essential enzymes that enables it to form 2 molecules of G3P, while student 7 thinks of the DHAP substrate as an enzyme. In both cases though, the students' mechanism whereby the 2 molecules of G3P are produced from 1 molecule of DHAP is unclear. Thus, they may recognise that the DHAP cannot be cleaved to form two 3-carbon G3P molecules, as it is a 3-carbon molecule itself. In this case, students may be trying to reason about reaction 5 (see Fig. 6.1), but are being misled by the representation used to show this reaction in the diagram and are, therefore, trying to draw on some conceptual knowledge to support what they think the diagram is depicting. The 8th quote illustrates a surface-level type reasoning difficulty, whereby some students that show the IR2 difficulty know, or can see, that 2 molecules of G3P enter the payoff phase of glycolysis (see Fig. 6.1), but are not describing exactly how they think this is achieved from 1 molecule of DHAP.

The above students showing the IR2 difficulty, may only be looking at reaction 5 (see Fig. 6.1) and therefore only see the reaction $\text{DHAP} \rightarrow \text{G3P}(2)$, instead of looking more closely at the diagram and trying to reason about reaction 5, from what they think happens in reaction 4. This would illustrate localised reasoning that has been proposed by Anderson *et al.* (e.g. 2002, pers. comm.), whereby students consider only one part of a metabolic process without considering the overall effects of that reaction on the entire process.

The incidence of the IR2 difficulty was calculated as 15.6% (14/90) from students' choice of option (c) to the probe in Fig. 6.4 and their supporting explanations. In interviews, none of the 15 student volunteers from this second year sample were found to hold the IR2 difficulty. Thus in order to investigate the possible sources of the IR2 difficulties, interviews with students manifesting these difficulties would be required (since students showing IR2 did not volunteer for interviews), in order to gain insight into how these students think 1 molecule of DHAP forms 2 molecules of G3P. By considering the mode of representation, i.e. Fig. 6.1 we may however, make suggestions as to the nature of the difficulties, be it conceptual, reasoning, diagram factor or an interaction between these factors (Schönborn *et al.*, 2003 & 2002) (Section 6.3.4).

6.3.2.3 Students' Correct Visualization of the Isomer Reaction

The following quotes illustrate some students' correct visualization of the reaction mechanism in reaction 5 of Fig. 6.1:

1. "Reaction 5: Dihydroxyacetone phosphate is converted by an enzyme into glyceraldehyde-3-phosphate \therefore now we have 2 molecules of glyceraldehyde-3-phosphate that is going to be used in the payoff phase of glycolysis and all the consequent products of the reactions to follow are going to be doubled." [Response to Fig. 6.2 about reaction 5]
2. "The Dihydroxyacetone phosphate is then converted to glyceraldehyde 3-phosphate in reaction 5 by another reversible reaction. After reaction 5 there are 2 Glyceraldehyde molecules for every molecule of Fructose 1,6-biphosphate that was originally converted." [Response to Fig. 6.2 about reaction 5]
3. "The second product of the cleavage of fructose 1,6, bisphosphate, that is Dihydroxyacetone phosphate is converted to its isomer glyceraldehyde 3-phosphate by the enzyme glyceraldehyde phosphatase [isomerase]. This then provides the two substrate molecules needed for the energy-producing section of glycolysis." [Response to option (b) in Fig. 6.4]
4. "Fructose 1,6-bisphosphate is cleaved to form one molecule of Glyceraldehyde-3-phosphate and 1 molecule of Dihydroxyacetone phosphate. In step (5) the 1 molecule of Dihydroxyacetone phosphate is converted to Glyceraldehyde 3-phosphate (1 molecule of)." [Response to option (b) in Fig. 6.4]
5. "Fructose 1,6-bisphosphate is cleaved to form 2×3 carbon compounds (glyceraldehyde 3-phosphate and dihydroxyacetone phosphate) but only glyceraldehyde 3-phosphate can be used for the rest of glycolysis \therefore dihydroxyacetone phosphate must be converted by enzyme at reaction (5) to avoid unnecessary waste of potential energy in the dihydroxyacetone phosphate molecule." [Response to option (b) in Fig. 6.4]

The above quotations illustrate how some students' correctly interpreted and visualized the chemistry of reaction 5 in Fig. 6.1, to represent how the molecule of DHAP is converted into its isomer G3P. These students also show that they were thinking of the stoichiometry of the reaction, since they made reference to the number of carbons involved in reaction 5 of Fig. 6.1 (Student 5), and/or that the products were doubled after the fructose 1,6-bisphosphate reaction (Students 1-3). Thus, these scientifically acceptable answers correspond to the propositional statements that were given in conjunction with the probe in Figs. 6.2 and 6.4 and thereby validate these probes (Chapter 3, Section 3.3.5).

Twenty-six students (26/90, 28.9%) showed a sound content knowledge or were able to correctly interpret or visualise the reaction mechanism at reaction 5 of Fig. 6.1. This was calculated from the number of students who correctly answered option (b) in Fig. 6.4, divided by the total number of students who answered this probe.

Interactions between some of the above students' correct answers will now be discussed in conjunction with students' correct understanding of reaction 4 (see Fig. 6.1) and students' who manifested the SR1, SR2, IR1 and IR2 difficulties, in the section that follows.

6.3.3 Interactions Between Students' Correct Responses, the SR and IR Difficulty Subclasses

Table 6.1, summarised the SR and IR classes and their respective subclasses of difficulty that were discussed in Sections 6.3.1 and 6.3.2 respectively. There was evidence that there may be an interaction between the difficulties, in that what students' visualised happening in reaction 4, might have influenced what they visualised happening in reaction 5 and vice versa (see Fig. 6.1). This could, for example, cause students to have different answers with respect to the total number of molecules of G3P that are formed as a result of reactions 4 and 5 (see Fig. 6.1). Thus, the incorrect diagrammatic reasoning about reaction 4 and/ or 5 (Fig. 6.1) by students leading to variations in the number of molecules of G3P students' visualised being formed from 1 molecule of fructose 1,6-bisphosphate, could influence students' understanding of the subsequent glycolytic reactions as depicted in reactions 6-10 of Fig. 6.1.

The following 2 interview extracts illustrate how the students' interpretation of reaction 4 influences the students' interpretation of reaction 5 in Fig. 6.1. The first student correctly identified the cleavage products in reaction 4 of Fig. 6.1, but held the IR1 difficulty with respect to reaction 5, as s/he was unsure about how s/he thought the molecule of G3P and DHAP reacted to form the final 2 molecules of G3P. This is shown by the following extract:

I: What do you think happens in reaction 5 [points to reaction 5 in Fig. 6.1]?

S: in reaction 5 glyceraldehyde 3-phosphate reacts with dihydroxyacetone phosphate to give you this [points to glyceraldehyde 3-phosphate (2)] but at the same time it can go back by that I mean that glyceraldehyde can react, can be converted to dihydroxyacetone phosphate, ja

I: ok, so when you say glyceraldehyde 3-phosphate and dihydroxyacetone phosphate react to form this glyceraldehyde 3-phosphate (2) what do you mean by react, what do you think happens there?

S: um, ok there will be an enzyme that will combine the two the two molecules together and it, and then some, ok an enzyme normally speeds up a reaction so I'd say that the two molecules would combine and give you glyceraldehyde 3-phosphate, ja that's what I think. You need glyceraldehyde 3-phosphate to combine with dihydroxyacetone to form phospho-dihydroxyacetone phosphate to form glyceraldehyde 3-phosphate, you can't like only have glyceraldehyde... oh no I'm actually lying here [giggles], no this is actually a confusing diagram, ja.

The above student correctly interpreted reaction 4 from Fig. 6.1 (i.e. fructose 1,6-bisphosphate forms 1 molecule each of G3P and DHAP). This student then used his/ her correct knowledge about reaction 4 and revealed a diagrammatic reasoning difficulty in

that s/he was trying to reason, using Fig. 6.1, about how G3P and DHAP would combine (as s/he may have been misled by the “+” sign) and then form G3P(2). In addition, the above student recognised that G3P could be converted back into DHAP, as this is a reversible reaction, but showed a difficulty when trying to describe the function of the enzyme.

The 2nd student, quoted below, held both the SR1 and IR1 difficulties (see Table 6.1) and, therefore, thought that only one molecule of G3P is formed from the cleavage of fructose 1,6-bisphosphate, which reacts with a DHAP molecule to form G3P(2). This student describes how she thought the DHAP and original G3P molecules were formed in reaction 4 (see Fig. 6.1), are combined and then split to form the final 2 molecules of G3P:

I: Describe what you think happens in reactions 4 and 5 [points to reactions 4 and 5, in Fig. 6.1]

S: ... four... [giggles] fructose can, is converted to glyceraldehyde.. glyceraldehyde 3-phosphate and then... it's combined with dihydroxyacetone phosphate to give you 2 glyceraldehyde 3-phosphate, assuming the two molecules are joined and then get cleaved to give 2 glyceraldehyde 3-phosphate

I: ok, so how do you think glyceraldehyde 3-phosphate(2) is formed?

S: the first glyceraldehyde 3-phosphate from, that is formed from the fructose [points to G3P in Fig. 6.1] whatever is joined onto the dihydroxyacetone and then cleaved, I think.

The above student provides some insight into how some students showing the IR1 difficulty might visualise the G3P and DHAP together forming the G3P(2) or 2 molecules of G3P (Fig. 6.1). The above diagrammatic reasoning about reaction 5 (Fig. 6.1), suggests that some students may think the G3P and DHAP combine and are then split to form the G3P(2). However, if G3P and DHAP combine, fructose 1,6-bisphosphate is formed. Thus, by “re-splitting” this molecule as suggested by the above student, 1 molecule each of G3P and DHAP are reformed, and not 2 molecules of G3P as reasoned by the above student.

In order to determine how many students had difficulties of the nature proposed by the above 2 interview extracts, students’ written responses were once again analysed. Thus, by comparing student responses to the probe in Fig. 6.3 (where students were required to draw what they thought the cleavage products of fructose 1,6-bisphosphate were), to their responses to the probe in Fig. 6.4 (about what they thought happened in reaction 5), what students’ collectively visualised happening in reactions 4 and 5 (see Fig. 6.1) could be determined. This comparison of student answers to the written probes in Figs. 6.3 and 6.4

was possible and valid, as these probes were found on the same tutorial test and some students' were correctly able to answer these probes (presented in Sections 6.3.1.3 and 6.3.2.3 respectively). Table 6.2, below, summarises the total number of students who had the different combinations of difficulties and correct answers, in equation form. The interactions between the difficulties have been indicated by the interaction code. The first part of the interaction code (e.g. SR1-) indicates what students' thought happened in reaction 4 and the second part (e.g. -IR1) what students' thought happened in reaction 5. In cases where students correctly answered the probes, this has been indicated by the code SAA (Scientifically Acceptable Answer) and placed in bold (see Table 6.2).

Table 6.2: Description and incidence of interactions of student difficulties and correct conceptions

Interaction Code	Students' Description of Reactions		Incidence of interaction
	Reaction 4 (Obtained from student responses to the probe in Fig. 6.3)	Reaction 5 (Obtained from student responses to the options of the probe in Fig. 6.4)	
SAA-SAA	Fructose 1,6-bisphosphate→ G3P + DHAP	DHAP → G3P	26.3% (21/80)
SR1-SAA	Fructose 1,6-bisphosphate→ G3P		2.5% (2/80)
SAA-IR1	Fructose 1,6-bisphosphate→ G3P + DHAP	G3P + DHAP → G3P(2)	35.0% (28/80)
SR1-IR1	Fructose 1,6-bisphosphate→ G3P		12.5% (10/80)
SR2-IR1	Fructose 1,6-bisphosphate→ G3P(2)		8.8% (7/80)
SAA-IR2	Fructose 1,6-bisphosphate→ G3P + DHAP	DHAP → G3P(2)	8.8% (7/80)
SR1-IR2	Fructose 1,6-bisphosphate→ G3P		2.5% (2/80)
SR2-IR2	Fructose 1,6-bisphosphate→ G3P(2)		3.8% (3/80)
			100.0% (80/80)

Key: **SAA**: Scientifically Acceptable Answer

SR1 and SR2: Split Reaction difficulties subclasses 1 and 2 (see Table 6.1)

IR1 and IR2: Isomer Reaction difficulties subclasses 1 and 2 (see Table 6.1)

The results (Table 6.2) show that 26.3% (21/80) of students who were able to correctly interpret reaction 4, were also able to correctly interpret reaction 5 in Fig. 6.1, as indicated by the interaction code SAA-SAA. However, a higher percentage of students (43.8%) who were able to correctly interpret reaction 4, were unable to correctly interpret reaction 5 as seen by the SAA-IR1 and SAA-IR2 interaction codes in Table 6.2. In this regard, thirty-five percent (28/80) of students who were correctly able to interpret reaction 4, were unable to correctly interpret reaction 5 as they thought the G3P and DHAP molecules were required to react with each other to form the G3P(2) (they showed the SAA-IR1 interaction in Table 6.2). In addition, a further 8.8% (7/80) of students, who were able to correctly interpret reaction 4 in Fig. 6.1, thought that the DHAP molecule was responsible for forming the G3P(2) as indicated in Table 6.2 by the SAA-IR2 interaction code.

Only 2 students (2/80, 2.5%) incorrectly interpreted reaction 4 while correctly interpreting reaction 5 (SR1-SAA, Table 6.2). This therefore, suggests that, even though students correctly interpret reaction 4, this did not affect whether or not they will be able to interpret reaction 5 (Fig. 6.1) as indicated by the SAA-SAA, SAA-IR1 and SAA-IR2 interaction codes. However Table 6.2 also indicates that if students incorrectly interpret reaction 4, there is only a very small chance that they will be able to interpret the subsequent reaction 5 (Fig. 6.1), as indicated by the interaction code SR1-SAA. In summary, students' incorrect interpretation or visualisation of a diagrammatic representation of a reaction in a metabolic pathway, will negatively affect whether they will be able to interpret/ visualise subsequent reactions in a biochemical process. However, if students are able to visualise a reaction correctly they may still have difficulty interpreting or visualising subsequent reactions due to various sources, as discussed in Section 6.3.4.

In considering our results from another angle, by looking at the equations in Table 6.2 explaining students' interpretations of reactions 4 and 5, we can calculate the total number of molecules of G3P that students might think or calculate are formed as a result of reactions 4 and 5 (Fig. 6.1). If we do this, students who correctly interpreted/ visualised reaction 4 and 5 in Fig. 6.1 (SAA-SAA) and students who thought that only one molecule of G3P was formed in reaction 4 but correctly visualised reaction 5 (SR1-SAA), are correct in that a total of 2 molecules of G3P are formed as a result of reactions 4 and 5 (see Fig. 6.1). In this way, we may suggest that a possible source of the SR1 difficulties is that students know that 2 molecules of G3P are formed and, therefore, use the diagram

incorrectly to reason about how they think these 2 molecules are formed and thus show the SR1 difficulty when interpreting reaction 4 (see Fig. 6.1). Alternatively, from the interpretations of reactions 4 and 5 (see Fig. 6.1) by students with the SAA-IR1, SR1-IR1, SAA-IR2 and SR1-IR2 interactions, these students might all think that a total of 3 molecules of G3P are formed. Furthermore, students with the SR2-IR1 and SR2-IR2 difficulty interactions suggest that they might think a total of 4 molecules of G3P are formed as a result of their interpretation of reactions 4 and 5 in Fig. 6.1. The implication of these calculations of the number of molecules of G3P students think are formed as a result of reaction 4 and 5 (Fig. 6.1), will have an effect on students' visualisation of the rest of the glycolytic pathway (reactions 6-10 in Fig. 6.1) in terms of stoichiometry.

Thus, this section, showing the interaction of student difficulties and correct answers pertaining to reactions 4 and 5 in Fig. 6.1, has revealed a method for investigating students' diagrammatic reasoning in cases where diagram elements are adjacent to each other. By comparing and contrasting students' answers and interpretations of successive parts of a diagram representing a metabolic process, we have shown how possible implications of student interpretation of one part of the diagram, influences another part of a diagram.

The emergence of the SR, IR and difficulty interactions have been presented and discussed in Sections 6.3.1- 6.3.3 respectively. Although we have suggested possible sources of these difficulties from students' responses to the various written probes and interview extracts, we applied a model proposed by Schönborn *et al.* (2003 & 2002), that investigates the factors that affect student interaction with diagrams, to our results in order to move closer to the possible sources and the interaction between 2 or more of the possible sources of the difficulties.

6.3.4 Possible Sources of the SR and IR Difficulty Subclasses

From the results presented in Sections 6.3.1- 6.3.2, the emergence of two classes of difficulties have been described, namely the SR and IR classes of difficulties (Table 6.1). The student responses to various written (see Figs. 6.2- 6.4) and interview probes suggested that the possible sources of the student difficulties may be due to the diagrammatic representation used to depict reactions 4 and 5 (Fig. 6.1) and because

students were not visualising the chemistry of these 2 reactions. In this regard, this section takes a look at the possible sources of the SR and IR difficulties in light of the model proposed by Schönborn *et al.* (2003 & 2002, Chapter 3, Section 3.3.8), i.e. in terms of the diagram factor, student's conceptual knowledge and the student's reasoning ability. Also included is various literature and suggested remediation and intervention strategies, that are further discussed in Section 6.4.

6.3.4.1 The Diagram Factor

A source of difficulty with interpreting scientific diagrams, that may be applicable to the SR and IR difficulties presented in this chapter, may be because some diagrams provide too little contextual information. This is because the user is assumed to be familiar with the conventions and symbols used, or due to the economy and clarity of the diagram (Lowe, 2001, pers. comm.). The results for the IR1 difficulty suggest that these difficulties may be due to the symbolism on the diagram. This is because some students stated that the “+” sign at reaction 5 (see Fig. 6.1) indicated that the G3P and DHAP were combined, while other students stated that reactions 4 and 5 (Fig. 6.1) were a confusing area of the diagram. In addition, the IR1 difficulties were seen to disappear from some students' responses (from comparing their written responses to interview extracts see Section 6.3.2.1) after they had been exposed to another diagram of the glycolytic pathway, which supports the use of multiple representations as a form of remediation or conceptual change. Furthermore, the confusing nature of reaction 4 may also be a source of the SR1 and SR2 difficulties, as students had difficulty with identifying the cleavage products of fructose 1,6-bisphosphate (see reaction 4, Fig. 6.1). This is because an addition or “+” sign in mathematics is a function, whereby 2 or more entities are combined or added to each other. In this regard, this may be why students with the IR1 difficulties thought that the G3P and DHAP were combined at reaction 5 in Fig. 6.1. However, in the English language a “+” sign also represents the word “and” i.e. G3P “and” DHAP are the products of reaction 4 (in Fig. 6.1). Therefore, if students showing the IR1 difficulty associated the word “and” with the “+” sign they may have interpreted it as G3P “and” DHAP form the G3P(2) (in reaction 5, Fig. 6.1). The IR2 difficulties may also be due to the diagram, as some students may have interpreted the DHAP molecule as being an intermediate that was fed into the glycolytic pathway, possibly indicated by the “+” sign, and it then formed the 2 molecules of G3P in reaction 5 (see Fig. 6.1). Furthermore, students with the SR difficulties may also have interpreted this ‘+’ sign (see reaction 4, Fig. 6.1) as the point at which DHAP was

added into the glycolytic pathway and therefore did not interpret the DHAP as being one of the cleavage products of fructose 1,6-bisphosphate.

In terms of remediation of the SR and IR difficulties, a key explaining the use of the symbols, as well as including the names and number of molecules of the cleavage products (G3P and DHAP) formed in the blue box on the left of reaction 4 (Fig. 6.1), might aid students in visualising the reaction mechanism. Although the diagram factor is strongly suggested to be a possible source of difficulty when students interpret reactions 4 and 5 in Fig. 6.1, some students may not be using the chemical structures on Fig. 6.1 as a visualisation tool for the reaction mechanisms. However, the representation used to depict these reactions, i.e. Fig. 6.1, may be a source of the visualisation difficulty for some students. Therefore, students should be cued to utilise the structures on Fig. 6.1 in order to determine if they are useful visualisation tools for the reaction mechanisms. In terms of visualisation, if students are not extracting the correct information out of the diagram to pass through their filter of prior conceptual knowledge, they will form scientifically unacceptable mental models of the depicted situation (Anderson *et al.*, 2003, pers. comm.). In order to remediate the SR and IR difficulties, which may be attributed to the diagram factor as discussed above, the use of multiple representations was shown to be effective in this study (Fig. 6.1 & 6.8, Section 6.3.2.1).

6.3.4.2 Students' Conceptual Knowledge

The quality of diagram design is a feature that will determine whether a diagram is effective at communicating information to the user, although the characteristics of the user should also be taken into account (Lowe, 1993b). In other words, the user's conceptual knowledge, as a diagram does not contain *all* the required information for effective use or interpretation (Cheng *et al.*, 2001). Therefore, the SR and IR difficulties may also have been influenced by the conceptual factor (Schönborn *et al.*, 2003 & 2002). This is possible as, although students were taught the chemistry of the glycolytic reactions, they may not have had the required level of prior knowledge about the reaction mechanisms to visualise the reactions. When applied to the definition of visualisation provided by Anderson (2003, pers. comm., Chapter 3, Section 2.3.2.1), this lack of prior knowledge would, therefore, constitute an inappropriate "filter" of prior knowledge through which students would pass their interpretations of a diagram, in order to form successful mental models of the depicted content. In this respect, Lowe (e.g. 1999 & 1996) has stated that abstract

diagrams depicting dynamic situations are more challenging for students that have a limited content knowledge, because this is what is required for the students to build an effective mental model of the depicted situation.

This apparent lack of content knowledge by the students, who showed the SR and IR difficulties, might have accounted for why they were unable to interpret the diagram in the manner that was intended. In this regard, Lowe (1993) has found that individuals, who build mental models of the depicted situation, based on the semantic characteristics of a diagram, are able to make inferences from the diagram that are connected to their content knowledge, not based only on the links between visiospatial elements of the diagram. A further indication that the conceptual factor may be an influencing factor for the source of the difficulties, is that the IR1 difficulties identified in students' written responses were seen to disappear in some students when they were interviewed. This might be because they had written a test on the glycolytic pathway and thus consulted their lecture notes. The students may then have acquired the required amount of conceptual knowledge in order to understand the representation (Fig. 6.1) of reactions 4 and 5. Furthermore, the interaction between the diagram and conceptual factors (Schönborn *et al.*, 2003 & 2002) is also illustrated by the disappearance of the IR1 difficulty after students had been exposed to another representation of the glycolytic pathway (Fig. 6.8) in conjunction with their course notes. This, therefore, illustrates how a certain minimum amount of conceptual knowledge is required for an individual to successfully use a diagram (Lowe, 1993).

6.3.4.3 Students' Reasoning Ability

Interview extracts illustrating the IR1 difficulties, showed how some students were using both diagrammatic reasoning and their conceptual knowledge, to visualise how they thought the G3P and DHAP combined to form the G3P(2). This illustrates an interaction between the conceptual and reasoning factors in the model of Schönborn *et al.* (2003 & 2002). In terms of students' reasoning ability, some students used the structures in blue and pink boxes, the arrows and even the "+" sign (Fig. 6.1) to try to reason or visualise about how they thought the G3P and DHAP formed the G3P(2). However, Lowe (1997) has stated that a diagram user needs to determine which elements of the diagram are related to each other, as their spatial arrangement in the diagram does not always indicate whether they are conceptually or functionally related. This suggests that students may have incorrectly reasoned about the nature of reactions 4 and 5 (Fig. 6.1). When

considering the role of students' conceptual knowledge, they were extensively taught the chemistry of the glycolytic pathway and the structures were provided on the diagram as reasoning and visualisation tools. Therefore in some cases, students who showed the SR and/ or IR difficulties may not have consulted the structures in order to interpret reactions 4 and 5, while others may have used them to incorrectly reason about reactions 4 and 5 (see Fig. 6.1).

This illustrates the interaction (Schönborn *et al.*, 2003 & 2002) between students' conceptual knowledge about the reactions, since they had been taught the chemistry, their reasoning ability, in that the structures were provided on the diagram to enable them to reason about reactions 4 and 5, and the mode of representation, that was still misleading students. This interaction was also shown during the construction of alternative conceptions and when conceptual change was driven by students' interpretation of a different diagram (Fig. 6.8) of the same phenomenon (Fig. 6.1) (Schonborn *et al.*, 2002).

The data suggests that many students might not have been visualising the chemistry of the glycolytic reactions appropriately. There is strong evidence to suggest that the diagram was the most influential source of the visualisation difficulty, as elements within the diagram were shown to confuse the students. Considering our results in light of the Schönborn *et al.* (2003 & 2002) model, we illustrate the interplay between the mode of representation (Fig. 6.1), the students' conceptual knowledge and the students' reasoning ability. All 3 of these factors may be contributing to the student difficulties in varying degrees of influence.

6.4 SUMMARY AND CONCLUSION

By readdressing our research questions as stated in Section 6.1, we summarise our major findings as follows:

1. Two major classes of difficulty (SR & IR, Table 6.1) were identified and classified as a result of students' using a textbook diagram (Fig. 6.1) to visualise 2 consecutive-linked glycolytic reactions;

2. Interactions between these difficulties and some students' correct conceptions were also observed (Table 6.2);
3. The possible sources of these visualisation difficulties were primarily attributed to the diagram factor, although interactions with students' conceptual knowledge and their reasoning ability (Schonborn et al., 2003 & 2002) were also found;
4. Our results have shown that the use of visiospatial characteristics and symbolism in diagrams may negatively influence students' visualisation of the chemistry of individual reactions in biochemical processes;
5. The presence of a different diagram (Fig. 6.8) of glycolysis aided students in correcting their previous difficulties and, thus, provided evidence in support of the use of multiple representations in instruction to foster appropriate mental model construction or promote conceptual;
6. In light of our results we suggest that caution be employed in diagram design and attention be paid to the use of symbolism and visiospatial features. This calls for the use of conventions in diagram design.

In terms of the sources of the SR and IR difficulties (Sections 6.3.1 and 6.3.2 respectively), the results revealed an interaction between the diagram, reasoning and conceptual factors proposed by Schönborn *et al.* (2003 & 2002). The results also illustrate that a minimum amount of content knowledge is required by an individual for them to successfully use a diagram (Lowe, 1993). Furthermore, that the primary source of the SR and IR difficulties seems to be the mode of representation (Fig. 6.1) that was used to illustrate the glycolytic reactions numbered 4 and 5. This is probably because the use of the arrows and "+" sign at reactions 4 and 5 in Fig. 6.1 were ambiguous and, therefore, misled students. This finding has important implications for diagram design as ambiguity should not be present and caution should therefore be taken when employing the use of multi-context symbols in diagrams. In terms of the students themselves, we found that many of them did not possess the required level of content knowledge to correctly interpret or reason about reactions 4 and 5. In addition, many of them did not use the chemical structures provided on the diagram (see Fig. 6.1) to aid the visualisation of the glycolytic reactions. Therefore, since students were shown to have difficulty in interpreting parts of Fig. 6.1, it was likely that they had difficulty using the diagram to visualise the chemistry. In addition to the diagram factor as a possible source for students' inability to successfully visualise the chemistry of reactions 4 and 5 (Fig. 6.1), some students were unable to use the wording in the boxes

adjacent to the reactions, the structures provided on the diagram, and the stoichiometry, to aid their visualisation of the reactions. Furthermore, in cases where students did use these additional visualisation tools, some of these students were shown to have difficulty with interpreting or reasoning with these tools.

Moving closer to the possible sources of the difficulties, enables us to make suggestions as to what intervention and or remediation strategies might be employed to circumvent these difficulties? In terms of improving students' conceptual knowledge, the students could be taught the chemistry of the individual reactions making up the process, which was done in the tutorials attended by students in our sample. However, since this content knowledge of reaction mechanisms did not circumvent the students' difficulties in this sample, we need to take a look at other possible sources of the difficulties such as the students' diagrammatic reasoning ability and the mode of representation itself (Schönborn *et al.*, 2003 & 2002). If the diagram factor (Schönborn *et al.*, 2003 & 2002) is a major source of the difficulty, then the students' ability to reason with the diagram, no matter how tuned their reasoning skills are, is likely to be hampered. However, if students are taught or prompted to relate the information they extract from the diagram to their conceptual knowledge, by forming links between their diagrammatic reasoning skills and their content knowledge, any misinterpretations that may arise from interacting with a particular diagram may be overcome by acquiring an extended content knowledge.

MacGregor and Slovic (1986) view effective diagrams as ones where only a small amount of information processing is required to transform the symbolic information. However, students conveyed in interviews that reactions 4 and 5 (Fig. 6.1) were confusing, which suggests that those particular reactions may not have been adequately represented and should be redesigned. In addition, the use of multiple representations is also useful as different representations can be used to highlight different aspects of the depicted situation (Seufert, 2003). In this regard, Fig. 6.8 was shown to aid students in correcting their previous difficulties that were a result of misinterpreting reactions 4 and 5 in Fig. 6.1. This, therefore, supports the use of multiple representations as an intervention and remediation strategy, which promotes conceptual change.

The results in this chapter, and in Chapter 5, illustrating that a diagram or elements within a diagram may mislead students, is an important area of research. This is extremely important because diagrams are used as primary teaching and learning tools in the field of biochemistry. As an extension of this work, we investigated student understanding of another diagram, that illustrates a biochemical process in the context of immunology. This investigation is reported on in Chapter 7 that follows.

CHAPTER 7

Students' Use of a Textbook Diagram to Visualise the Complement Pathways

7.1 INTRODUCTION

The immune system is a complex network of integrated processes that function to protect the body against infection and disease (e.g. Matzinger, 2002). The great number of diverse processes involved in this system (the integration of which is seldom clear) is a major reason for why students find immunology so conceptually complex and difficult to understand and visualise.

Only a limited amount of research has been done on student visualization and understanding of biochemical processes in the content area of immunology. For example, Schönborn *et al.* (2002) identified 3 major classes of difficulties that 2nd- and 3rd-year biochemistry students had with the interpretation and visualization of a textbook diagram of the tertiary structure of immunoglobulin G (IgG), while Simonneaux (2000) used interviews to investigate high school students' conceptions of the immune system and its function, as part of a larger study on biotechnology education. Alexander *et al.* (1995) also conducted a study in immunology education, in which they used premedical students' performance profiles to investigate the interrelationship and interplay of knowledge, interest and recall. In addition, other immunology education literature has focused on games that allow high school students to, for instance, act out immunology scenarios (Bealer & Bealer, 1996), to play a board game called "ImmunoScenarios" that is designed to reinforce concepts learned in the classroom (Taylor & Jackson, 1996), or to make paper models of antibodies (Baker & Moore, 1998). The rest of the immunology education literature is rather diverse in nature and includes articles that for instance focus on: students writing entries into a journal and critiquing scientific papers (Cannon, 1990); introductory laboratory experiments to demonstrate the immune response to students using cockroaches and venom (Yurkiewicz, 1993); cost-effective and simple experiments including immunodiffusion using goat serum albumin and anti-bovine serum albumin (BSA)

(Tayyab *et al.*, 1993); affinity chromatography of immunoglobulin G (IgG) (Ferreira *et al.*, 1996); enzyme-immunodotting using cow and goat milk (Inda *et al.*, 1998); enzyme-linked immunosorbent assays (ELISA) using BSA and anti-biotin peroxidase (Anderson & McNellis, 1998); and, teaching catalytic antibodies to undergraduate students using organic chemistry (Shulman *et al.*, 1999).

The complement system, the focus of this chapter, has been reported by Scroferneker *et al.* (1995) to be very difficult to teach to medical students. The reasons they give are that the topic requires abstract thought and it is difficult for students to understand fragment sequences of the protein cascade and thus the whole system. Therefore, they proposed a board game that requires student groups to answer closed-ended questions about the classical, alternative and post-C3b complement system. After playing this game, 80% of fourth-semester medical students' were able to creatively visualise the complex functioning of the complement cascade (Scroferneker *et al.*, 1995). However, this study did not measure student conceptual understanding, and board games are time-consuming and not always practical in bigger classes, thus the conventional method of teaching or lecturing is often employed.

This chapter investigates student visualisation of the complement pathways represented by a textbook block-word flow diagram (see Fig. 7.1), to identify any conceptual, reasoning or diagram related difficulties. The following research questions were thus formulated: (1) Do students have difficulty visualising enzyme reactions of the complement system as represented by a diagram?; (2) If so, are these difficulties more influenced by the conceptual, reasoning or diagram factors (based on a model by Schönborn *et al.*, 2003 & 2002)?; (3) What is the nature of expert and student understanding of the relationship between the 2 complement pathways?

7.2 METHODS

A detailed account of methods employed in this chapter can be found in Chapter 3.

7.2.1 The Study Group

This study was conducted with 20 students (and with 27 students in a subsequent year), studying 3rd-year biochemistry who had previously completed a module on immunology in their second year. Interviews were conducted with 10 student volunteers from the above group of 20. Five immunology experts were also consulted for their opinion on complement pathway functioning of which 2 responded (as discussed in Section 7.3.2.1). These experts all held at least senior-lecturing positions at their respective universities with the one expert (Roitt) being the author of the textbook from which the diagram (Fig. 7.1) used in this investigation was extracted.

7.2.2 Description of the Diagram and Relevant Biochemistry

The diagram of the complement system (Fig. 7.1) was taken from the prescribed immunology textbook for the course that our sample of students was enrolled in (Roitt, 1997, pg 24). The same diagram was also found in the 2001 edition of this textbook (Roitt & Delves, 2001). For convenience, a larger copy of Fig. 7.1 can be seen in Appendix D.

Roitt and Delves (2001) describe complement as the 20 plasma proteins that form part of the humoral (or soluble) immune response to invading microbes that aids the cellular immune response. The complement system is composed of the classical and alternative complement pathways, which although composed of different proteins, essentially have the same function. Figure 7.1 is a diagrammatic representation comparing the classical and alternative complement pathways. The complement pathways are enzyme cascades of protein reactions where the product of one reaction serves as the catalyst for the next reaction (Chapter 4, Table 4.1). Proteolytic cleavage is required to activate some complement molecules, as these molecules are pro-enzymes, or zymogens. These complement components that have enzymatic activity are designated by over-bars in the diagram. The over-bars, which seem to be idiosyncratic to the textbook author, Roitt, are represented as stars in the text (e.g. *C3bBb). Suffix letters indicate that cleavage has occurred. For example, C3b is the cleavage product of C3. Some cleavage products of the

complement reactions have been omitted from the diagram probably for the sake of simplicity. For example, C3 is cleaved into C3b and C3a, but (confusingly) C3a is not shown in Fig. 7.1. In all but one case, the “a” indicates the smaller of the 2 cleavage products. The exception is C2 where C2a is larger than C2b (e.g. Roitt & Delves, 2001). Students were instructed on this complement terminology or symbolism and in particular, the $C3 \rightarrow C3a + C3b$ reaction, as it is the central reaction to both the classical and alternative complement pathways.

Both pathways result in the independent cleavage of the C3 protein into C3b via the catalytic action of their own structurally distinct convertase enzymes, namely $C3bBb$ in the alternative pathway and $C4b2b$ in the classical pathway (Fig. 7.1).

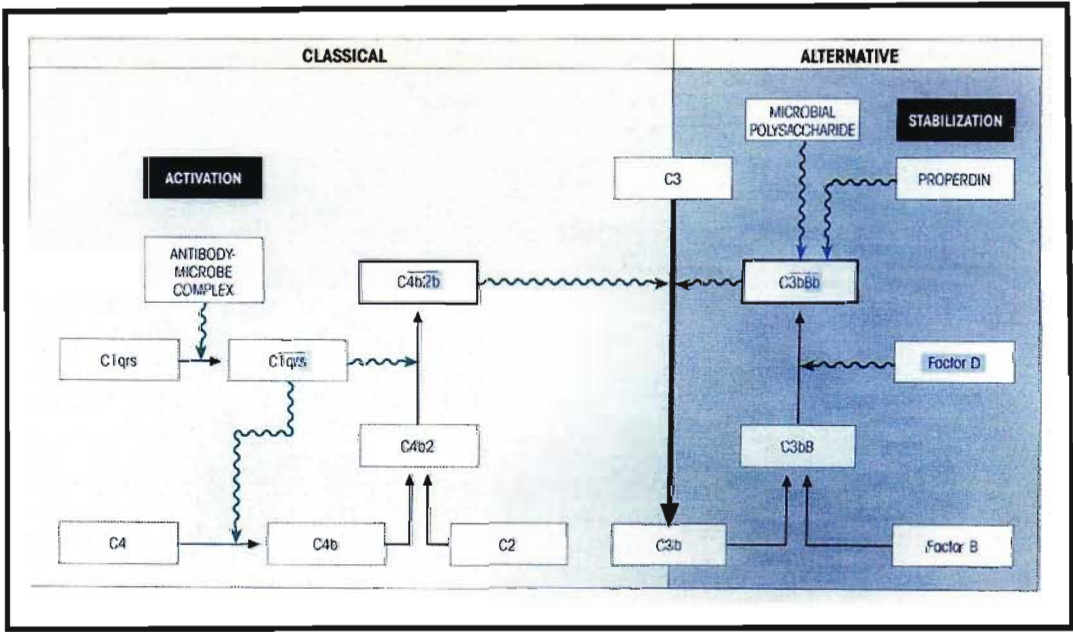


Figure 7.1: Comparison of the classical and alternative complement pathways.
(Roitt, 1997, Reprinted by permission of Blackwell Science, Inc.).

Roitt and Delves (2001) describe the post-C3b pathway (not shown in the diagram) leading to *lysis* of the invading microbe as being the same in the classical and alternative pathways. *Lysis* is the process whereby a pore is formed in the microbial cell membrane by the membrane attack complex (MAC) causing the microbe to fill with liquid and consequently burst. In this regard, once either pathway has formed C3b, the C3b binds to the C3 convertase that cleaved the C3 molecule on the surface of the microbe to form a C5

convertase (i.e. $*C3bBb + C3b \rightarrow *C3bBbC3b$ in alternative pathway and $*C4b2b + C3b \rightarrow *C4b2bC3b$ in classical pathway). However, it is not certain whether C3b, produced by the classical complement pathway, is able to be used in the alternative complement pathway as discussed in Section 7.3.2. The C5 convertase then cleaves C5, which is the first complement molecule involved in the MAC which consists of the complement proteins C5-C9. Although the MAC is not shown in Fig. 7.1 above, the students were instructed on this process using diagrams from the same textbook that they had access to.

The major physiological effects of the complement system are, therefore, the opsonisation of microorganisms, i.e. the binding of C3b to the surface of microbes enhancing phagocytosis of the microbe by phagocytes that have C3b receptors on their surfaces; lysis (as described above) and cellular activation and inflammation i.e. the cleavage products C3a and C5a are anaphylatoxins (Roitt & Delves, 2001). More detailed information on individual processes within the complement system is provided in Section 7.2.4, as part of the propositional knowledge statements to some of the research probes used in this study.

7.2.3 Analysis and Classification of Student Responses

Inductive analysis (McMillan and Schumacher, 1993) (Chapter 3, Section 3.3.7) of student responses to various weekly written probes (see Figs. 7.2, 7.5- 7.8), interview probes and student-generated diagrams, was used to investigate students' visualisation of complement reactions and any related conceptual and reasoning difficulties. Students' written explanations of their choice(s) to 2-tier multiple-choice type probes were analysed to assess whether they had a sound understanding of what was being tested, which was especially important when these probes may have had more than one correct answer. The research framework of Grayson *et al.* (2001) was used to classify difficulties, at levels 1-3, according to how much insight we had into the nature of each difficulty (see Chapter 3, Section 3.3.2). The possible sources of the difficulties are considered in Section 7.3.3 in light of a model proposed by Schönborn *et al.* (2003 & 2002), which investigates the factors that affect student interaction with diagrams. The analysis and interview process used in this chapter is described in detail in Chapter 3, Sections 3.3.7 and 3.3.4 respectively.

7.2.4 Written and Interview Probes: Design and Rationale

All probes were given to students post-instruction and included a black and white computer scanned copy of Fig. 7.1, and a coloured version in the subsequent year's study. The full caption of the diagram was omitted (as seen in Fig. 7.1) in order to investigate student reasoning about the complement cleavage reactions (see EC difficulty in Section 7.3.1). Instruction on the complement pathways included a detailed discussion of the chemistry of the processes in the two pathways.

In order to determine whether students' responses to the various written probes (in Figs. 7.2, 7.5- 7.8) were scientifically acceptable answers, or constituted a difficulty, we composed a list of propositional statements for each probe and had these validated by an immunology expert in the School of Molecular and Cellular BioSciences, University of Natal. The propositional statements are given in conjunction with each probe in this section, and are elaborated on in Section 7.3 where student difficulties, correct answers and variations of the propositional statements are discussed. The students would require a degree of content knowledge to answer probes in Figs. 7.3 and 7.4, that tested their understanding of the relationship between the 2 complement pathways. This is because the fate of C3b leading to the membrane attack complex (MAC), is not shown in the diagram. All probes were given to the students shortly after instruction.

Initially, a free-response type probe ("Describe everything you think this diagram is meant to represent or show") was given to the students to allow them to reveal their difficulties. Student responses to free-response type probes informed the development of semi-focused type probes that were developed to focus on aspects or concepts in the diagram, but not to lead the students. The semi-focused type question seen in Fig. 7.2 below, was designed to investigate students' understanding of the key individual reaction within the complement pathways.

Use the diagram provided to answer the following question and support your answer(s) with an explanation.

Explain how C3b is formed.

Propositional Statement (based on Roitt & Delves 2001):
C3 is independently cleaved to form C3b and C3a by one of two structurally distinct convertases depending on which pathway was activated, i.e. *C3bBb in the alternative and *C4b2b in the classical complement pathway. (This reaction illustrates that lower case letters i.e. "a" and "b" represent that cleavage has occurred).

Figure 7.2: Semi-focused type probe given to the subsequent year's group of students and propositional statements

Subsequent probes given to the students were two-tier multiple-choice questions that focused more specifically on the nature of each difficulty (Treagust, 1988; see Chapter 3, Section 3.3.3). This type of question (see Figs. 7.3- 7.8) honed in on difficulties, or differences in understanding, that had already been revealed in student answers to previous written probes as well as other suspected difficulties (that had not yet been revealed), by requiring students to choose an answer and motivate for their choice. Incidence of student difficulties, revealed by these two-tier multiple-choice probes, were calculated and recorded.

Use the diagram provided to answer the following question and support your answer(s) with an explanation.

Once C3b has been produced by the classical complement pathway:

(a). C3b binds to Factor B to form C3bB

(b). C3b binds to *C4b2b to form a C5 convertase

(c). C3b binds to a microorganism

(d). Both a. and c. occur

(e). Both b. and c. occur

a b c d e (circle your selection(s))

Explanation.....

Figure 7.3: Two-tier multiple-choice type probe (Treagust, 1988) given to the students

Use the diagram provided to answer the following question and support your answer(s) with an explanation.

Read the following statements and choose the one(s) you think best describe(s) the relationship between the classical and alternative complement pathways:

(a). The classical pathway is dependent on the alternative pathway

(b). The alternative pathway is dependent on the classical pathway

(c). The alternative pathway requires intermediates produced by the classical pathway

(d). The two pathways function totally independently of each other

(e). Both (a) and (b)

a b c d e (circle your selection(s))

Explanation.....

Figure 7.4: Two-tier multiple-choice type probe (Treagust, 1988) given to the students

Use the diagram provided to answer the following question and support your answer(s) with an explanation.

The formation of C3b from C3 via the action of *C3bBb occurs as follows:

(a). C3 is activated by *C3bBb to form C3b

(b). C3 binds to *C3bBb to form C3b

(c). C3 is enzymatically cleaved by *C3bBb to form C3b

a b c (circle your selection(s))

Explanation.....

Propositional statements:

-The scientifically accepted answer is option (c), as this is the end result of the reaction. C3a is the other cleavage product of this reaction

-Option (a) is also possible as the *C3bBb does act as an activator

-Option (b) is also correct as an enzyme-substrate (*C3bBb-C3) complex is formed in the reaction mechanism but this is an intermediate step

-(Students would therefore have to state that cleavage was occurring in their explanations to choices (a) & (b)).

Figure 7.5: Two-tier multiple-choice type probe (Treagust, 1988) given to the students and propositional statements

Use the diagram provided to answer the following question and support your answer(s) with an explanation.

In the alternative complement pathway, C3 yields:

- (a). C3b
- (b). C3b and Factor B
- (c). C3a and C3b
- (d). C3a, C3b and Factor B

a b c d (circle your selection(s))

Explanation.....

Propositional statements:

- The scientifically accepted answer is option (c) (which means that option (a) is also partially correct, since C3a is not represented in the diagram):
- C3 is cleaved to form C3b and C3a by *C3bBb.

Figure 7.6: Two-tier multiple-choice type probe (Treagust, 1988) given to the students and propositional statements

Use the diagram provided to answer the following question and support your answer(s) with an explanation.

When Factor D participates in the activation of C3bB to yield *C3bBb:

- (a). Factor D cleaves off a portion of the C3bB molecule to yield *C3bBb
- (b). Factor D provides energy to convert C3bB to *C3bBb
- (c). Factor D adds another molecule to C3bB to yield *C3bBb

a b c (circle your selection(s))

Explanation.....

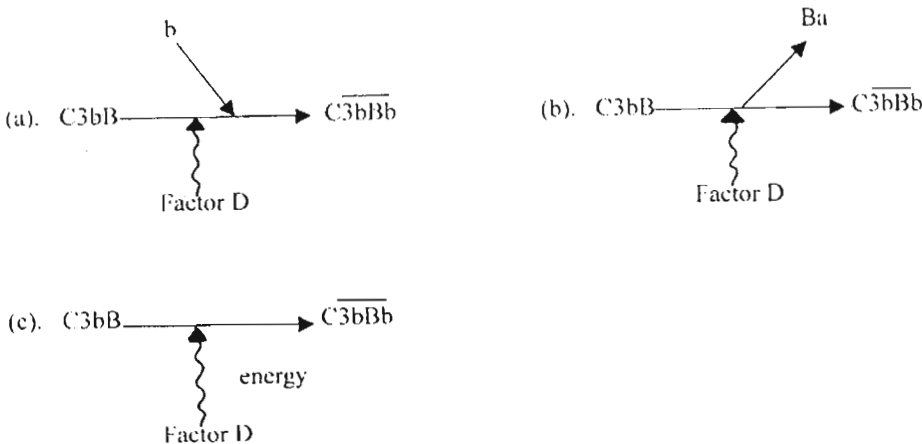
Propositional statements:

- The scientifically accepted answer to this question is (a):
- Factor D is a serine proteinase specific for C3bB and responsible for cleaving the Ba protein portion off the parent C3bB molecule resulting in the active enzyme *C3bBb.
- (This reaction illustrates that the lower case letter i.e. "b", represents that cleavage has occurred).

Figure 7.7: Two-tier multiple-choice type probe (Treagust, 1988) given to the students and propositional statements

Use the diagram provided to answer the following question and support your answer(s) with an explanation.

Indicate which of the following processes (a, b or c) correctly describes the conversion of C3bB into *C3bBb, in the alternative complement pathway?



a b c (circle your selection(s))

Explanation.....

Propositional statements:

- The scientifically accepted answer is option (b):
- Factor D is a serine proteinase specific for C3bB and responsible for cleaving the Ba protein portion off the parent C3bB molecule resulting in the active enzyme *C3bBb.

Figure 7.8: Two-tier multiple-choice type probe (Treagust, 1988) given to the students and propositional statements

Interviews were conducted to gain greater insight into the nature of each identified difficulty and its possible source. This powerful technique enabled the classification of difficulties at level 3, or partially established (Grayson *et al.*, 2001). A detailed account of the interview process is given in Chapter 3 (Section 3.3.4).

7.3 RESULTS AND DISCUSSION

In Section 7.2 we mentioned that a black and white computer scanned copy of Fig. 7.1 was given to the students with all written probes. This was done for convenience, as many copies were required. Interviews, however, showed that some students preferred the coloured version simply as it was “more interesting”, which subsequently led to the use of coloured diagrams, as they appeared in textbooks, for subsequent studies.

One major class of visualization difficulty was identified from the inductive analysis (McMillan & Schumacher, 1993) of student responses to the probes in Figs. 7.2- 7.8, namely, the enzyme cleavage (EC) difficulty. The EC (enzyme cleavage) difficulty, together with the incidence and classification level (Grayson *et al.*, 2001), as well as student correct conceptions, will be dealt with in Sections 7.3.1. In Section 7.3.2, evidence is presented for the confusion about whether the two complement pathways are chemically linked, which led to the exposure of a deficiency in expert knowledge. In both Sections 7.3.1 and 7.3.2 we discuss the nature and possible sources of difficulty, or interpretation, based on a model (Schönborn *et al.*, 2003 & 2002, Chapter 3, Section 3.3.8) investigating factors that affect student interaction with diagrams.

7.3.1 Student Visualisation of Enzyme Cleavage (EC) in the Complement Pathways

The enzyme cleavage (EC) difficulty was suspected (Level 2) by the present author from experience with the diagram (Fig. 7.1), and the representations or symbolism used to describe the complement pathways. The EC difficulty stems from students not being able to visualise that the complement pathways are a series or cascade of cleavage reactions. Thus, some students view the addition of a lowercase letter (e.g. “b”) as molecule addition from one step to the next in the complement pathways, therefore showing a symbolism problem. The EC difficulty was classified at level 3 on the framework of Grayson *et al.* (2001) and is discussed in Section 7.3.1.1 that follows. Section 7.3.1.2 then discusses the possible sources of the EC difficulty based on a model by Schönborn *et al.* (2003 & 2002), followed by illustrations of students’ correct responses in Section 7.3.1.3.

7.3.1.1 The EC Difficulty

Although in the diagram (Fig. 7.1), *C3bBb has an extra “b” when compared to C3bB, it is the complement protein C3bB that is cleaved to form *C3bBb and Ba rather than vice versa. Thus, in complement terminology, or symbolism, the upper case letters therefore indicate addition of a molecule and the lower case letters depict the result of a cleavage reaction (see Section 7.2.2 for more detail). Therefore, the EC difficulty stems from the misunderstanding of both confusing symbolism, and the chemistry of how complement molecules/ proteins are formed. The EC difficulty is illustrated by the following quotes in response to the various written probes indicated in brackets:

1. “This is because C3bB is not broken down in any way, a molecule is added.” [Response to option (c) in Fig. 7.7]
2. “Factor D adds the b molecule [to] C3bB to yield *C3bBb.” [Response to option (c) in Fig. 7.7]
3. “Factor D facilitates \perp [the] addition of b to C3bB thus activating it to *C3bBb.” [Response to option (a) in Fig. 7.8]
4. “...C3bB will need another b to make C3bBb [*C3bBb].” [Response to option (a) in Fig. 7.8]
5. “Factor D provides the catalytic mechanism of the pathway. Factor B provides the extra protein constituent to the complex.” [Response to option (a) in Fig. 7.8]

The students quoted above state that the “b” molecule has been added to C3bB to form *C3bBb which may be because they have noticed that there is an extra “b” on *C3bBb when compared to C3bB. In this regard, Students 1- 4 quoted above do not state where they think the “b” comes from, that they think is added to C3bB, to form *C3bBb. However, the 5th student suggests that Factor B provides the “b” protein to C3bB. This is incorrect because, although Factor B does contribute the “B” to C3b to form C3bB, it does not contribute the second “b” which is a result of the cleavage of C3bB by Factor D to form *C3bBb and Ba. The 3rd and 5th student also view Factor D (the enzyme in this reaction) as facilitating the addition of this “b” molecule. This confusion may be due to the representation used in option (a) of the probe in Fig. 7.8. Thus, the above preliminary data on the EC difficulty suggests that it may stem from students not visualising the chemistry of the complement reactions, as well as from the confusing symbolism used to represent that cleavage has occurred. It would seem, therefore, that students are required to have a certain degree of visual literacy with respect to the symbolism and the chemistry represented by the symbolism, used in Fig. 7.1.

The following 3 extracts are all taken from an interview with the same student. In the first part of the interview, the student correctly describes that C3 is cleaved to form C3a and C3b:

I: Compare the size of C3 to C3b [points to C3 and C3b in Fig. 7.1 respectively], which is bigger?

S: C3b should be smaller because C3 cleaves and form C3b and C3a

The student was then asked to compare the sizes of C3bB and *C3bBb, and thought that *C3bBb was bigger as there are more letters (which represent molecules) and that the second "b" in *C3bBb might indicate that there was molecule addition:

I: so what is that second small "b" [points to second "b" of *C3bBb in Fig. 7.1]?

S: I don't know, [mumbles] maybe it shows that there was a binding of some form

I: ok, so let's compare the size of C3bB to *C3bBb [points to C3bB and *C3bBb in Fig. 7.1 respectively], what do you think is the difference in size there?

S: *C3bBb is bigger

I: why do you think it's bigger?

S: because there's more letters

When questioned on a different reaction, the same student stated that s/he thought that *C1qrs activated C4b2 to bind to "b" but did not know where this "b" molecule came from:

I: what is *C1qrs?

S: ...it's a complement, it's I can't think of the name, but it's an activator

I: Ok so what does *C1qrs do to C4b2 [points to C4b2], to get *C4b2b [points to *C4b2b]?

S: it's activates C, C4b2 to bind to "b"

I: ok, where do you think this "b" [points to second "b" of *C4b2b] comes from?

S: ...I'm not sure

The above interview excerpt shows that this student knows cleavage has occurred in the reaction $C3 \rightarrow C3b$, but has not transferred this knowledge to the reactions involving *C1qrs and Factor D in the complement pathways. This could be due to the student's memory, i.e. the student has learnt that C3 is cleaved into C3a and C3b, but does not recognize that the 'b' indicates a portion of the parent molecule that has just been cleaved. This would constitute a terminology or symbolism problem where the student does not recognise the use of the upper and lower case letters in the complement pathways and thereby uses this knowledge to reason about the nature of other complement reactions.

The following student provides further evidence for the EC difficulty. In the first part of the following interview extract the student shows that s/he knows that C3 is cleaved to form C3a and C3b:

I: ok, let's compare C3 with C3b now [points to C3 and C3b in Fig. 7.1 respectively], what is the difference between those two molecules?

S: ...according to how they've been drawn I can say there's no difference in size

I: what tells you that there is no difference in size, from the way they're drawn?

S: like since this has been drawn as a rectangle, both, these two rectangles [points to boxes in which C3 and C3b have been written in Fig. 7.1], they look of the same size

I: ok

S: but what I can say is that C3b is smaller than C3, because C3 was broken down into C3b and C3a

However, when the same student was asked to explain the role of *C1qrs in the formation of *C4b2b, s/he describes that *C1qrs adds the "b" molecule to C4b2 but is unsure of the mechanism. The first part of this interview extract is also interesting as it shows how the student uses surface-level features of a diagram (i.e. the boxes wherein the complement molecules are written), to answer the question about the sizes of molecules. This is consistent with work done by Lowe (e.g. 2001, pers. comm.) where he found novices' interpreted diagrams based on their visiospatial characteristics and not semantics. However, the above student's content knowledge superseded the perceptual or visiospatial information that s/he was interpreting from the diagram. The above interview extract also suggests a possible remediation technique for the EC difficulty: if the boxes in which the complement molecules are written were of different sizes, this may help students visualise that cleavage is occurring (see Section 7.3.1.2).

The next quote demonstrates that the student only recognised cleavage in the reaction $C4 \rightarrow C4b$ in the classical complement pathway because s/he was taught it in lectures and remembered it. However, when probed deeper on the terminology used in the complement pathway, the student could still not reason with the terminology that the lower case "b" represents a cleavage product, and reverted back to the EC difficulty:

I: ... but how do you know that from C4 to C4b [points to $C4 \rightarrow C4b$ in Fig. 7.1] cleavage has taken place?

S: um, that's why I said that it doesn't help much if you don't know the theory behind it

I: ok, what is the difference, you said earlier the difference between C4 and C4b [points to C4 and C4b], what is the difference between those two molecules?

S: C4a has been removed from C4 to make C4b

I: ok, so how do you know from the reaction of C4 into C4b, how do you know that cleavage has occurred in this reaction [points to $C4 \rightarrow C4b$]? What does the terminology tell you?

S: ...uh...

I: how did you know that C4 had been cleaved into C4a and C4b [points to $C4 \rightarrow C4b$ in Fig. 7.1]?

S: that's what the theory says

I: Can you not tell that from the diagram?

S: No, I don't think you can, no you can't, unless of course you see the C4a molecule somewhere on the side, in the diagram

I: Ok, but what do you think about that 'b' molecule [points to 'b' in 4b]?

S: the b, the small 'b'?

I: ja

S: ...eya...it shows something has happened, but it doesn't say exactly what

I: ok

S: it could have been the small 'b' added to the C4 protein

The above interview extract suggests that this student may have rote learnt some of the cleavage reactions of the complement pathways but is not familiar with the terminology used. S/he thinks that the only way of determining that cleavage has taken place is if the cleavage products are added into the diagram, or from content knowledge, and although this student is aware of some of the cleavage reactions of the complement pathway, s/he doesn't reason with the terminology or symbolism. Therefore, this student illustrates how surface-level interpretation prevails over a deeper interpretation of the diagram that uses complement terminology, even when the student is probed more deeply on some concepts of the complement pathways.

The EC difficulty, was also shown by the following student who stated that both Factor D and *C1qrs are responsible for adding a "b" molecule. Thus, this student thinks that *C3bBb is bigger than C3bB because it has an extra "b". However, we surmise that this student is unsure how this 'b' got there, as illustrated by the following quote:

I: ok, so can you tell me at the molecular level, what is the difference between those two molecules [points to C3bB and *C3bBb in Fig. 7.1 respectively]?

S: um, the convertase one [points to *C3bBb] it will be bigger than the the C3 one below [points to C3bB]

I: why do you say that?

S: because it has a extra 'b' on it

I: ok, now how do you think that extra 'b' [points to second 'b' of *C3bBb] gets there?

S: ...well I don't know that

I: can you draw what you think is happening in this reaction with Factor D then?

S: [silence while student draws Fig. 7.9, below]...



Figure 7.9: Student generated diagram of the reaction involving Factor D in the alternative complement pathway

In contrast to the above student, who placed the “b” in the diagram not knowing its origin, the following quotation shows how some students may have tried to reason with the complement pathway terminology:

I: ok, so take a look at this molecule over here [points to *C3bBb in Fig. 7.1], this *C3bBb, where do you think all those letters come from?

S:...um.. they come from the the different molecules that are designated by by a similar letter, a similar order same letter for instance, C3b combines with Factor B and that's where the capital 'B' [points to B of C3bB] comes from, from Factor B. And then it combines with Factor D but because there is a conformational change in the molecule it now becomes small 'b', rather than big 'B'

I: ok so where does that small 'b' [points to second 'b' of *C3bBb] come from?

S: it comes from Factor D

I: can you explain how it gets there from Factor D?

S: ...[long silence] it's simply the addition of Factor D to C3bB [points to C3bB→*C3bBb in Fig. 7.1]

I: so what is that small 'b' [points to second 'b' of *C3bBb]

S: it is it is Factor D, but in a different shape, Factor D is no longer exactly like Factor D because it combines with C3bB, that Factor D joins to it but in a slightly different shape that's why it's not 'D' but small 'b'

Thus, the above student knew that upper case letter addition (e.g. C3bB from C3b) indicated molecule addition had occurred. However, when this student was asked what the second small “b” on *C3bBb meant, this student reasoned that it also indicated molecule addition since a letter had been added. In conjunction with this, the student suggested that Factor D had undergone a conformational change when binding to C3bB to form *C3bBb, probably because there was no “b” in Factor D, and therefore this student may have thought that the “D” in Factor D had changed to a “b”. Therefore, in contrast to the other interview extracts presented in this section, the above student did reason using complement terminology, although the student’s reasoning was incorrect in terms of which letters indicated molecule addition.

The student responses to Fig. 7.7 option (c), and Fig. 7.8 option (a), showed incidence of 22.2% (4/18) and 33.3% (5/15) for the EC difficulty, respectively. Based on the student responses to the written probes in Figs. 7.7 and 7.8, and the interview extracts, the EC difficulties were reclassified from level 2 or suspected difficulties to level 3 or partially established, on the framework of Grayson *et al.* (2001).

In summary, the EC difficulties may stem from both students’ chemistry knowledge and their lack of understanding of the symbolic conventions used to represent the complement

proteins. This is suggested by the fact that students with this difficulty thought that the more letters or “symbols” in a compound, the “bigger” it is. We suggest, in this case that surface-level reasoning was occurring in terms of the terminology used in the complement system. Chemistry, at both first and second year level, is a prerequisite for biochemistry. The cleavage products of the reactions are omitted in the diagram, probably for the sake of simplicity. Students would therefore have to have prior knowledge that cleavage is occurring and be able to transfer this knowledge to other reactions, in order to understand Fig. 7.1. The following section takes a look at possible sources of the EC difficulty in more detail according to the model of Schönborn *et al.* (2003 & 2002).

7.3.1.2 Possible Sources of the EC Difficulty

The EC difficulty (discussed above) was attributed to students not understanding the symbolism used to represent the cleavage reactions in the complement system. However, student responses presented in Section 7.3.1.3 below, illustrate that 93.8% (15/16) of students knew C3 was cleaved to form C3b, while 94.7% (18/19) of students knew that C3 formed both C3a and C3b. Therefore this suggests that students showing the EC difficulty were familiar with the chemistry of this process, but unable to use the required terminology to reason about reactions. The exception is the key $C3 \rightarrow C3b$ reaction, in the complement system.

According to the model of Schönborn *et al.* (2003 & 2002, Chapter 3, Section 3.3.8), the EC difficulty might also stem from a lack of conceptual knowledge. This may be because students with this difficulty rote learnt that C3 is cleaved to form C3b and did not link this to the complement system terminology. They did not have the prior or content knowledge about the chemistry of the complement reactions and the symbolism used in the diagram to represent the reactions. In this regard, diagrams depicting dynamic situations have proved challenging for those students with limited content knowledge (as discussed in Chapter 2, Section 2.3.3.3), because they place high demands on the students’ cognitive processing of the diagram, and content knowledge is required in order for the student to build an effective mental model of the situation (Lowe, 1999 & 1996). In our case, the students’ lack of content knowledge about the terminology of the complement system, may therefore have contributed to the EC difficulty.

We also believe that a degree of interaction between factors, proposed by Schönborn *et al.* (2003 & 2002), is illustrated with respect to the EC difficulty. For example, as a remediation strategy, if the blocks wherein the complement molecules were written (see Fig. 7.1) were of different sizes (mentioned earlier), and the cleavage products of the reactions were included in Fig. 7.1, this may have helped cue students to interpret the diagram, that cleavage reactions were occurring. This potential remediation strategy lends itself to the possibility that this EC difficulty may also be attributed to the diagram factor (Schönborn *et al.*, 2003 & 2002). Other authors such as Holliday (1990), have also suggested that the elements within a diagram should be in proportion to avoid confusion, while Phillips and Quinn (1993) have suggested that more familiar symbols should be employed in flow diagram design if the limitations of the flow diagram are that the user is unfamiliar with the complexity and symbols that are used. Both of these suggestions may be applied to the design of Fig. 7.1.

In the following section, we present some students' correct responses to the same written probes that revealed the EC difficulty.

7.3.1.3 Students' Correct Visualisation of Enzyme Cleavage

As dealt with in Chapter 3 (Section 3.3.5), in order to show that a probe is both valid and fair, some students in the sample should be able to correctly answer the probe. Quotes 1-4 below illustrate students' correct understanding of C3 cleavage and quotes 5 and 6 students' correct conceptual understanding of C3bB cleavage:

1. "C3 is enzymatically cleaved by *C3bBb which is a C3 convertase which gives C3a and C3b." [Response to (c) in Fig. 7.5]
2. "The answer should be (c) but according to the diagram there is only C3b." [Response to (a) in Fig. 7.6]
3. "C3a is not reflected (shown) on the diagram provided but C3 does yield both C3b and C3a." [Response to (c) in Fig. 7.6]
4. "C3b is formed via the classical or alternative pathways from the cleavage of C3 into C3b and C3a by C3 convertase of which there are two forms: (1) C4b2b produced by the classical pathway of complement activation (2) C3bBb produced by the alternative pathway of complement activation." [Response to Fig. 7.2]
5. "C3bBb is a fraction/ part of C3bB, thus Factor D must cleave a portion of C3bB to yield *C3bBb." [Response to (a) in Fig. 7.7]
6. "C3bB is cleaved by Factor D to the activated product *C3bBb. Ba is released as the cleavage product." [Response to (b) in Fig. 7.8]

When considering the responses to option (c) in Fig. 7.5, as illustrated by quote number 1, it was concluded that 15 out of 16 students (93.8%) were aware that C3 was actually

cleaved to form C3b. Furthermore, 7 of these students (47.7%) stated that C3 was cleaved into both C3a and C3b, although C3a was not mentioned in the probe in Fig. 7.5. In light of this data, we wanted to test how many students, who *were* aware that cleavage was occurring, knew or remembered from lectures that C3 was cleaved to form both C3a and C3b. To achieve this, the exact mechanism of the reaction (i.e. “cleavage”) was omitted from the question (in Fig. 7.6) so as not to hamper any future results on the actual mechanism of the reaction. Quotes 2 and 3 represent the 94.7% (18/19) of students who correctly responded to this probe i.e. knew the C3 was cleaved to form both C3a and C3b, irrespective of their choice (i.e. either (a) or (c) respectively) to the probe. In addition, quote 2 is an example of how 2 students may have been misled by the diagram as some of the cleavage products were omitted (i.e. C3a in the case of this probe). Thus, although they knew C3 forms C3a and C3b (quote 2), they were not sure how this knowledge corresponded to the diagram. In contrast, quote 3 represents that some students (who also chose option (c) in response to the probe in Fig. 7.6) seem to realise that C3 forms both C3a and C3b, although C3a has been left out of the diagram, probably for the sake of simplicity.

Quote 4 is an example of students’ correct responses in the duplicate investigation of whether students enrolled in the subsequent year’s immunology course (Section 7.2.1), were aware that cleavage was occurring in the complement reaction $C3 \rightarrow C3a + C3b$, post-instruction. These students were doing the same course that those students reported in this chapter did. Analysis of these latter student responses showed that 89% (24/27) of students in this subsequent year’s class, did recognize that C3 was cleaved to form C3b, as illustrated by quote 4.

In terms of the cleavage of C3bB, quote 5 is an example of students’ correct responses to the probe in Fig. 7.7, option (a). However, none of these students named the protein portion Ba as the cleavage product, possibly because it is not included in Fig. 7.1. In contrast, quote number 6 shows this student knows, or read from the figure (see Fig. 7.8), that Ba is the portion of C3bB that is cleaved off to form *C3bBb.

From the results of the probes in Figs. 7.5, 7.6 (93.8%, 15/16) and 7.3 (89%, 24/27, subsequent year’s batch of students) it can be seen that most students are aware that cleavage is occurring in the reaction $C3 \rightarrow C3a + C3b$, post-instruction. This may be

because it is still “fresh” in their mind or because they have rote learnt this reaction that is taught in detail in their immunology course. From the analysis of students’ correct responses to the probes in Fig. 7.7 and 7.8, it was calculated that fewer students (33.3%, 6/18) knew that C3bB was cleaved by Factor D to form *C3bBb. In this respect, 20.0% (3/15) of students in response to the probe in Fig. 7.8 recognised that cleavage was occurring when a representation of the reaction was presented to them in the form of option (b). Although fewer students were aware cleavage was occurring in the reaction $C3bB \rightarrow *C3bBb$, the probes testing for this knowledge of complement cleavage reactions were still valid and fair, as illustrated by students’ correct responses to the probes in Figs. 7.7 and 7.8.

Thus, based on the conclusion that most students were aware C3 was cleaved to form C3b, and the possibility that students may understand the terminology used in the complement reactions, students should therefore be able to transfer this reasoning to work out the nature of the other reactions in the complement pathways. This was however found to be untrue as the EC difficulty (Section 7.3.1.1) and its possible sources (Section 7.3.1.2) illustrated.

The following section presents data that illustrates how a diagram, through design features, may mislead students.

7.3.2 Expert and Student Understanding of the Relationship Between the Complement Pathways

Before investigating student understanding and visualisation of the complement pathways (Section 7.3.2.2), it was important to research expert knowledge of the system, in particular the propositional knowledge represented by the diagram. Since the diagram (Fig. 7.1), in the current author’s opinion, depicted the 2 complement pathways functionally linked via the $C3 \rightarrow C3b$ reaction, it was considered important to establish whether experts agreed with this assertion. This led to an in-depth investigation into expert understanding of this issue, the findings of which are presented in the following section. In addition, Section 7.3.2.2, presents some student interpretations of the diagram as supporting evidence for the unclear representation of the current knowledge in the diagram with respect to this issue.

7.3.2.1 Expert Propositional Knowledge about Complement Pathway Functioning

Although current literature on the complement system focuses on various aspects of complement pathway functioning such as, the role of complement in bacterial pathogenesis (e.g. Bohlson, *et al.*, 2001) and complement activation by various microorganisms and antibodies (e.g. Rosas *et al.*, 2002 & Zhang & Kozel, 1998), a literature search revealed no explicit evidence as to whether the classical and alternative complement pathways are functionally linked or not. In view of this, we decided to further investigate this issue by e-mailing 5 experts for their opinion, 2 of whom responded.

An e-mail discussion with Professor Ivan Roitt, the author of the textbook from which the diagram for this chapter (Fig. 7.1) was taken, is shown in Fig. 7.10.

"Dear Prof Roitt,
 "A fellow student and myself were looking at a figure in your textbook "Essential Immunology" (9th edition, figure 2.3, pg 24) on the complement pathway. We were having a debate as to whether it would be possible for C3b produced by the classical pathway to be used/ fed into in the alternative pathway?

Considering that the reactions are very localized (occurring on the surface of a microbe), I thought that it was very unlikely. The half-life of C3b is also short and if C3b found in solution was in fact produced by the classical pathway, would this be an insignificant amount and how would one be able to tell whether it was in fact produced by the classical pathway? My fellow student says that it should be possible.

We were wondering whether you could please help us with this question if time permits.
 Thank you.
 Tracy Hull."

The following is the reply obtained from Professor Ivan Roitt to the above e-mail:

"Dear Tracy,
 I love to hear students worrying about the depth of their subject. To determine whether C3b in solution was produced by the 'classical' pathway, one would introduce the antibody-coated bacterium into a normal animal and then into one lacking any one of the early classical components such as C4 or C2: Whether factor B would combine to C3b on the surface of an opsonized bacterium, I cannot answer. If it did so, then factor D might cleave it to form a convertase. Best of luck!, Ivan Roitt."

Figure 7.10: Correspondence From Professor Ivan Roitt pertaining to the question of whether C3b produced by the classical complement pathway could be used in the alternative complement pathway

The response in Fig. 7.10 shows clearly that Professor Ivan Roitt is unsure whether C3b produced by the classical complement pathway can enter the alternative pathway. He further suggests that the classical pathway be "inactivated" and an antibody-microbe

complex, which is responsible for activating the classical complement pathway, injected into the animal to determine whether Factor B binds to C3b. However, experimental data shows that this experiment may not be as simple as suggested, as mycobacteria have been shown to activate both the classical and alternative complement pathways in the absence of specific antibodies (Bohlson *et al.*, 2001). This is in contrast to what was previously reported i.e. that the classical complement pathway is activated by antibody binding to the microorganism (e.g. Roitt, 1997). In addition, both the classical and alternative pathway have been shown to be activated by antibody-dependant mechanisms (e.g. Zhang and Kozel, 1998), although the alternative pathway may also be activated in the absence of specific antibodies (Xu *et al.*, 2001).

In an attempt to gain greater insight into the question of whether C3b, produced by the classical complement pathway, could be used in the alternative complement pathway, i.e. suggesting that the 2 complement pathways may be linked at this point, another expert (Dr Tony Hugli) was e-mailed the question seen in Fig. 7.11 together with Professor Ivan Roitt's response (Fig. 7.10).

"Can C3b produced by the classical complement pathway be fed into the alternative complement pathway?"

"I don't believe that these systems are compartmentalized to the extent that C3b decides which pathway it joins once generated. The issue would seem to depend on the extent of amplification in the alternative pathway provided by having excess C3b around. The tick-over from factor D cleaving factor B is always there to produce very low levels of C3b. When the levels of C3b are enhanced by any mechanism that stabilizes the Bb-C3b complex, one would expect to see some additional alternative pathway activity. I don't know that the question can be answered by depletion of the classical pathway factors, because the resultant generation of excess metastable C3b by the classical pathway enzymes is needed if the alternative pathway is to be recruited. Somewhat of a Heisenberg uncertainty situation in terms of direct testing. A better test might be a sensitive test for Bb generation during the classical pathway activation process. This would only answer if more factor B is being converted, but would not answer the alternative condition of better utilization of the Bb formed by the intrinsic tick-over process. If the increase in C3b in the environment enhances Bb effectiveness, then I don't know how one would answer the question. Perhaps if the contribution from the alternative pathway was a significant part of the total, then measuring the extent of C3 consumption with and without Factor B in the system would give an answer assuming proper controls were used."

Figure 7.11: Correspondence from Dr Tony Hugli pertaining to the question of whether C3b produced by the classical complement pathway could be used in the alternative complement pathway

Initially, Dr Hugli indicates that he thinks it unlikely that the classical and alternative complement pathways function independently, but that they may be linked (Fig. 7.11). However, he then goes on to disagree with the experiment suggested by Roitt in Fig. 7.10, and suggests an alternative experiment, the outcome of which he is unsure, as he hasn't investigated this issue empirically.

The conclusions that may be drawn from the above evidence is that neither expert is sure whether C3b, produced by the classical complement pathway, can be used in the alternative complement pathway, thereby forming a link between these 2 pathways at the point where Factor B combines with C3b in the alternative pathway. Furthermore, neither expert can agree on how to experimentally verify this issue. In addition, we have found no literature with experimental evidence that states and shows that C3b, produced by the classical complement pathway, is used in the alternative pathway. Clearly, future experimental research is required to address this deficiency in expert knowledge while, in the mean time, any diagrammatic representation of the 2 complement pathways should not suggest that the 2 pathways are either linked or not linked functionally.

To confirm that the diagram (Fig. 7.1) was misleading to students, their interpretations of the diagram are presented in Section 7.3.2.2 below.

7.3.2.2 Student Interpretation of the Diagram

This section presents quotes that illustrate, firstly, how some students interpret the 2 complement pathways as being chemically separate, and secondly, how other students interpret the 2 pathways as being chemically linked.

The following quotes in response to a semi-focused and a multiple-choice type probe (indicated in brackets), illustrate that some students interpreted the diagram as representing 2 pathways that were not linked:

1. "The only thing (step) that the pathways have in common is the cleavage of C3 into C3b and C3a, which both pathways do independently of each other. The two pathways are activated differently- the classical is activated by antibody-microbe -complex and the alternative by microbial polysaccharides. The classical has its own C3 convertase (*C4b2b) that is different from that of the alternative pathway (*C3bBb)." [Response to option (d), Fig. 7.4]

2. [Selected option (e), Fig. 7.3] "C5 convertase is formed to continue the complement activation and C3b binds to a microorganism to allow the continuation of the C3 loop to produce more C3b."

The 1st student states that both processes, although activated differently, both cleave C3 into C3b, which is a common step, but the processes do it independently of each other. Also, student 2 reveals that s/he knows about the MAC complex and C3b's function as an opsonin.

Further evidence that students were interpreting the 2 pathways to be separate in Fig. 7.1 is provided in the following interview extract. The student quoted below shows how s/he understands the 2 complement pathways to be composed of different molecules and where the classical complement pathway is activated by the antibody-microbe complex, the alternative pathway is activated through the stabilisation of the *C3bBb on the microbial surface:

I: why are there two pathways?

S: ...it's because they are different it's, it's..

I: ok, how are they different?

S: they're different in the C4, the alternative pathway doesn't have that C4 and the classical has C4, and because the enzymes, the splitting enzymes [points to both *C3bBb and *C4b2b], are different. The whole pathway is different

I: ok, so now why do you think the body developed two pathways with different molecules in them?

S: it's because they're activated in different ways

I: ok, tell me about that

S: mm, in the alternative pathway, the microbe is, the microbial, they form a complex, the microbial polysaccharide it stabilizes the conver...enzyme convertase to cleave the C3, but in the classical it's the antibody-microbe complex that activates

I: ok, so what happens to C3b [points to C3b] when it's produced by this classical pathway [waves hand over classical pathway]?

S: it goes to form the membrane complex.

In the last line of the above extract, the above student explicitly showed his/ her interpretation, that the pathways are not linked. This student stated that the C3b formed in the classical complement pathway, is used in the membrane attack complex (MAC, that causes lysis of invading microorganisms, see Section 7.2.2) and not in the alternative pathway.

The number of students who interpreted the 2 complement pathways to be separate was calculated as 27.3% (5/19) from students' explanations to their choices of options (b), (c) and (e) of the probe in Fig. 7.3; and 42.1% (8/19) from student explanations to their choice

of (d) in Fig. 7.4. In contrast to the above quoted students, quotes illustrating how some other students linked the 2 complement pathways are presented below.

The following quotes suggest that students form a link between the classical and alternative complement pathways at the point at which C3b, formed by the classical complement pathway, binds to Factor B of the alternative pathway:

1. [Selected option (a), Fig. 7.3] "From the arrows on the diagram on[ly] a occurs."
2. [Selected (b), Fig. 7.4] "Because *C4b2b of the classical pathway together with *C3bBb of the alternative pathway activates C3 (which belong to both pathways) to form C3b which in turn in the presence of Ca^{2+} and Factor B form[s] C3bB in the alternative pathway."
3. "Classical and alternative pathway both act on C3 but the product from C3 break down influence[s] the function of alternative pathway since it is required to combine with Factor B to form C3bB, hence *C3bBb." [Response to option (c), Fig. 7.4]

The quotes above suggest that these students may be combining the two complement pathways (see Fig. 7.1) at the point at which *C3bBb from the alternative, and *C4b2b from the classical complement pathways, are shown reacting with C3 in the diagram, and subsequently, where C3b combines with Factor B in the alternative pathway. Furthermore, the 1st and 2nd student suggest that they may be using diagrammatic reasoning to interpret that the classical pathway C3b enters the alternative pathway. In addition, quotes 2 and 3 show that although students chose different options (i.e. either (b) or (c), in other cases (e)) to Fig. 7.4, some of these students still interpret that Fig. 7.1 represents the link between the 2 complement pathways. Interviews allowed us to gain more insight into the nature of this interpretation.

The following 2 interview extracts are clear examples of diagrammatic reasoning showing how Fig. 7.1 led these students to link the two complement pathways, at the point at which C3b produced by the classical pathway combines with Factor B of the alternative pathway:

1. I: What do you see happening to that C3b [points to C3b in Fig. 7.1] from the classical pathway [points to classical pathway in Fig. 7.1]?
 S: uum, well the C3b would move into the alternative pathway
 I: is that from your prior knowledge or is that what you're deducing from the diagram?
 S: just what I'm deducing from the diagram.
2. I: Describe this step for me from the *C4b2b to the C3b [points to arrows leading from *C4b2b to C3 and C3 → C3b in Fig. 7.1], just those reactions there
 S: *C4b2b, from the classical pathway, acts on C3 and then C3 gets broken down into C3b
 I: ok, so what happens to that C3b now?

S: C3b now binds to Factor B from the alternative pathway and forms a complex of C3bB [points to C3bB]...

The above interview extracts indicate that the diagram may be the major source of the students' interpretations that the 2 complement pathways are linked, as they were shown to reason, using Fig. 7.1, about the fate of C3b once produced by the classical pathway.

The very high incidence of this latter interpretation, whereby students were found to link the 2 complement pathways, were calculated from the probe in Fig. 7.3 options (a) and (d) as 68.4% (13/19), and from the probe Fig. 7.4, as 53% (10/19).

In summary, the student data clearly shows that the diagram was misleading some students into believing that the 2 pathways are linked and others that they are not linked. This is in contrast to what the diagram is intended to show- a mere comparison of the 2 pathways, since experimental work is still required to establish the functional relationship of the pathways. The possible sources of these interpretations can be suggested, as dealt with below according to the model proposed by Schönborn *et al.* (2003 & 2002, Chapter 3, Section 3.3.8).

7.3.2.3 Possible Sources of Students' Misinterpreting the Diagram

Schönborn *et al.* (2003 & 2002) have shown that student difficulties can often be more attributed to the representation mode itself, i.e. the actual visiospatial characteristics of the diagram, rather than reasoning mechanisms and conceptual knowledge, used to understand the diagram. Furthermore, Winn (1993 & 1991) has said that diagram elements are unconsciously grouped or separated by the diagram user and the user does not consider the meaning of the symbols and why they are placed or connected in certain areas. In terms of this perceptual grouping of diagram elements, Lowe (e.g. 1996 & 1993) has also found that novices grouped abstract diagram elements according to visiospatial characteristics, such as whether they were visually distinctive, close in proximity or part of the same visiospatial pattern. These reports are applicable to our study since the complement pathways, for comparative purposes (see Fig. 7.1), are situated next to each other and the complement proteins are visually similar with respect to the boxes in which they are represented. In addition, the 2 pathways form part of the same visiospatial pattern when the arrows from C3 → C3b, which subsequently combines with Factor B, are followed, even though the C3b may have been formed from the classical complement pathway.

Although McNamara (1986) has stated that a boundary line between two closely situated diagram elements was sufficient to have these elements assigned to different groups, or pathways in our case, we believe Fig. 7.1 is misleading. This is because the arrows showing the combination of C3b and Factor B in the alternative pathway (see Fig. 7.1) were interpreted by students as being the point where C3b produced by the classical pathway entered the alternative complement pathway. Another possible reason for students' interpretation of the 2 pathways as being chemically linked, may be as a result of the placement and presentation of the two pathways side by side, as unconscious organization of diagram elements occurs according to their proximity (Kosslyn, 1989). The common $C3 \rightarrow C3b$ reaction indicated with a bold arrow (see Fig. 7.1) might also be a diagram factor misleading students, as heavier marks and brighter colours are said to be given more attention in flow diagrams (e.g. Kosslyn, 1989).

It would seem, therefore, that 2 entirely separate diagrams are required for the instruction of the complement system, with Fig. 7.1 being used as a summary to allow the comparison of the classical and alternative complement pathways. However, a high proportion (52%, 14/27) of students still linked the classical and alternative pathways even though the pathways were separated by a space in a modified diagram of Fig. 7.1 (not shown in this dissertation). This was in a subsequent study using a different sample of 3rd year biochemistry students (Hull *et al.*, 2002). One of the reasons that students may still have interpreted the 2 complement pathways as being chemically linked in this latter study, was suggested to be because students had already been exposed to the unmodified Fig. 7.1 during instruction on the complement system.

There is, therefore, strong evidence that the diagram factor (Schönborn *et al.*, 2003 & 2002) was a major source of students' suggesting that the 2 complement pathways are chemically linked. These findings, highlight the importance of diagram design in biochemistry.

7.4 SUMMARY AND CONCLUSION

Three research questions were presented in Section 7.1, namely, (1) Do students have difficulty visualising enzyme reactions of the complement system as represented by a diagram?; (2) If so, are these difficulties conceptual, reasoning or diagram in nature (based on a model by Schönborn *et al.*, 2003 & 2002); (3) What is the nature of expert and student understanding of the relationship between the 2 complement pathways? We feel these questions were answered in this chapter, as summarised below.

The EC difficulty presented in this chapter shows students do have difficulty visualizing the complement reactions as represented by a diagram (Fig. 7.1). Therefore, we suggest that it should not be assumed that flow diagrams showing overviews of biochemical processes, will necessarily improve student understanding of the overall and individual processes, especially if they are used as a primary teaching and learning tool. Furthermore, the EC difficulty shows that the use of symbolism in diagrams can confuse students as to the nature of the chemical substances and processes occurring in a biochemical system. The possible sources of the EC difficulty were, therefore attributed to a possible interaction between the conceptual, reasoning and diagram factor (Schönborn *et al.*, 2003 & 2002). In this regard, students who knew about the key complement reaction ($C3a \rightarrow C3b$), may not have recognized the use of complement symbolism and therefore did not reason about the nature of the other complement reactions, thus illustrating the interaction between the conceptual and reasoning factor (Schönborn *et al.*, 2003 & 2002). A further interaction between the diagram factor was suggested through diagram design parameters, discussed in Section 7.3.1.2.

The diagram used in this study (Fig. 7.1) was, according to the diagram caption, clearly intended to show a comparison between the classical and alternative complement pathways, rather than to indicate any common chemical reaction between each pathway. The student data, however, suggested that students were divided in their interpretation of the diagram. Thus, some students suggested chemical links and others no link between the 2 pathways. Therefore, this study is an example of how a diagram might not be interpreted in the same way as intended by textbook authors. The deficiency in expert knowledge regarding whether the 2 complement pathways are chemically and thus functionally linked,

revealed an area where more laboratory research is required to determine whether C3b, produced by the classical complement pathway, can enter the alternative pathway.

In conclusion, the inability of students to visualise the chemistry of the complement reactions constitutes further evidence that students have difficulty in the visualisation of biochemical processes in terms of their spatial arrangement within cells (Chapter 5) and with respect to their chemistry (Chapters 6 & 7). Furthermore, the nature of these visualisation difficulties may be attributed to any one, or a number, of influencing factors (Schönborn *et al.*, 2003 & 2002). Each of the above chapters (5- 7) has suggested possible remediation and intervention strategies for the difficulties that were identified. In this regard, the General Discussion in the next chapter suggests further diagram design guidelines as a remediation strategy. This fits one of the primary focuses of this dissertation; the use of diagrams as visualisation tools.

CHAPTER 8

General Discussion

The overall objective of this dissertation was to investigate students' use of diagrams for the visualisation of biochemical processes, and to identify the nature and potential sources of students' conceptual, reasoning and diagram-related difficulties revealed during the visualisation process. In pulling together the results of all the studies carried out and reported on in this dissertation (Chapters 4- 7), we revisit our research questions that were presented in Chapter 1, namely:

1. What is experts' understanding of the distinguishing functional features of linear, cyclic and cascade biochemical processes and what recommendation can be made for the description of these processes in textbooks and the literature?
2. How do students' visualise the spatial arrangement of biochemical processes occurring within cells?
3. How do students' visualise the chemistry of the individual reactions in a biochemical process, represented by a textbook diagram?
4. What influence do textbook representations of biochemical processes have on students' ability to visualise these processes?
5. Do students show any visualisation difficulties and, if so, what is the nature of the difficulties?
6. What are the possible sources of these visualisation difficulties?
7. What are the implications of this research for improving student visualisation of biochemical processes?

By addressing the above research questions in Chapters 4- 7, we achieved a number of results that we believe are novel in the context of biochemistry. These results will be revisited and synthesised in this discussion, where we subsequently propose a model for the process of visualisation, and suggest possible avenues for future research.

Question 1 (see Chapter 4), was addressed first as we considered it important to establish our understanding of the meaning of linear, cyclic and cascade biochemical processes before investigating students' visualisation of these processes in cells (Chapter 5). In this

regard, the experts' responses to the questionnaire revealed an extensive list of generic features and 2 distinguishing functional features for each process, to which we added a further feature, that the processes are depicted as conventional representations (e.g. cyclic processes are represented in circles). Their results suggested to us, that a list of both distinguishing functional features and generic features for each process would be far more useful to students and researchers, than only the distinguishing features. In considering the generic features, there was a lack of consensus between experts and textbook authors as to which feature is characteristic of which process. Thus this study illustrated that the functional characteristics of these biochemical processes are not well established and that, in contrast to one expert's view (Chapter 4, Section 4.4), it is necessary to define them, especially as students were later shown to have difficulty with visualising these processes (Chapter 5). In addition, for communication purposes, biochemists have divided biochemical processes into functional units or segments of a network of metabolic systems, thereby further highlighting the need to clarify this "language" of metabolism. Towards this end, information on the functional features of linear, cyclic and cascade biochemical processes (Chapter 4, Table 4.1 & 4.2), has already been incorporated into a 2nd-year biochemistry metabolism course (Anderson, 2003, pers. comm.).

Another example of the lack of consensus among biochemistry experts was revealed by the results reported in Chapter 7. In this case, 2 experts were uncertain as to whether the 2 complement pathways were chemically linked or not. They, therefore, suggested that further laboratory research is required to investigate the existence of any functional relationship between the 2 complement pathways. Thus, both of the above studies, that involved gathering experts' opinions (Chapter 4 & 7), revealed areas where further research is required to clarify and expand the theoretical content knowledge of biochemistry as a subject. These are, therefore, good examples of where science can benefit from science education research.

Research questions 2- 7 were addressed in the studies reported on in Chapters 5- 7. The results of these studies revealed a number of student difficulties with the visualisation of biochemical processes in terms of the spatial arrangement of processes in cells (Chapter 5), and the chemistry of individual reactions (Chapter 6 & 7). In results reported in Chapter 5, some students showed difficulties with the visualisation of linear, cyclic and cascade biochemical processes *in vivo*. Conventionally, textbook representations of biochemical

processes represent linear processes in a straight line, cyclic in a circle and cascade in a step-wise form. In this respect, an expert that was part of the sample in one of our studies (Chapter 4, Section 4.4) remarked that s/he did not think it necessary to define the characteristics of these processes, as the everyday words (i.e. linear, cyclic and cascade) adequately described these processes. Our results, however, showed that some students were found to literally interpret the words and mode in which these processes are conventionally represented in textbooks, to be the manner in which their molecules are spatially arranged in the cell (e.g. linear processes occur in a “straight line”). This literal interpretation was, more often than not, found to be the first superficial characteristic some students used to classify processes and would constitute a surface-level reasoning difficulty.

In terms of the chemistry of biochemical reactions, students revealed that they had difficulty visualising 2 consecutive-linked glycolytic reactions (Chapter 6) and the enzyme-catalysed cleavage reactions of the complement pathway (Chapter 7), represented by 2 different flow diagrams. With respect to glycolysis, 2 major classes of visualisation difficulty (see Chapter 6, Table 6.1), namely the SR (split reaction, Section 6.3.1) and IR (isomer reaction, Section 6.3.2) classes were identified. The SR difficulty class was comprised of 2 subclasses, the SR1 and SR2 subclasses that were classified at level 2 on the framework of Grayson *et al.* (2001), and had incidence of 16.3% (13/80) and 12.5% (10/80), respectively. In contrast, the IR difficulty class was also comprised of 2 subclasses, namely the IR1 subclass classified at level 3 (Grayson *et al.*, 2001) with an incidence of 55.6% (50/90), and the IR2 subclass which was classified at level 2 (Grayson *et al.*, 2001) and had an incidence of 15.6% (14/90). These SR and IR classes of difficulties, stemmed from students misinterpreting the symbolic manner in which the textbook diagram (Fig. 6.1) represented the 2 consecutive-linked glycolytic reactions (i.e. use of “+”, arrows and arrangement of intermediates), as well as their inability to visualise the chemistry of these reactions. Furthermore, in some cases students could not interpret either glycolytic reaction, while other students could correctly visualise either the first reaction but not the second, or vice versa (Chapter 6, Table 6.2). This constituted strong evidence for how student interpretation of the symbolism in one part of a diagram can influence their interpretation of related diagram elements. In this regard, by comparing students’ mental models of these 2 consecutive-linked glycolytic reactions, we identified a method of investigating students’ diagrammatic reasoning (Chapter 6, Section 6.3.3). In

conclusion, since the 2 glycolytic reactions are consecutive-linked, these results constitute strong evidence for how a misleading symbolic feature of a diagram can affect students' interpretation of a related feature in the same diagram.

The above inability of students to correctly visualise the chemistry of biochemical reactions, when provided with a textbook diagram, was also found in the complement investigation (Chapter 7). The students in this study (Chapter 7, Section 7.3.1) showed difficulty visualising the chemistry of various complement reactions symbolically represented in the diagram (Fig. 7.1), by various combinations of upper and lower case letters of the alphabet. This led to the identification and classification, at level 3 (Grayson *et al.*, 2001), of one major class of difficulty (EC, enzyme cleavage) that had an incidence of 22.2% (4/18)- 33.3% (5/15), according to the probe used to calculate it.

In terms of our results we, therefore, had a common thread running across our investigations into student understanding and visualisation of biochemical processes (Chapters 5- 7): students showed difficulty visualising biochemical processes, which seemed to be strongly influenced by the symbolic nature of the diagram representing the chemical reactions and overall process. When considering the possible sources of these student visualisation difficulties mentioned above, an interaction between the 3 factors proposed by Schönborn *et al.* (2003 & 2002) was suggested in the various chapters (Chapters 5- 7). By moving closer to these possible sources in each chapter, we were able to suggest possible remediation and intervention strategies, applicable to the difficulty source. This is readdressed below.

Schönborn *et al.* (2003 & 2002) proposed that 3 factors influence a students' ability to interpret a diagram namely, the students' prior or conceptual knowledge, their reasoning ability, and the diagram or mode of representation itself. Firstly, to address the conceptual factor, our results on students' visualisation difficulties confirm those of Lowe (1993), in that a certain minimum level of content knowledge is required by an individual in order for them to successfully use a diagram. In support of this, content or prior knowledge is reported by a number of authors (e.g. Cheng *et al.*, 2001 & Lowe, 1994 & 1993) to be important in interpreting a diagram and subsequently constructing an appropriate mental model of the depicted situation. This role of content knowledge in diagram interpretation has been considered in detail in various sections of the literature review (Chapter 2,

Sections 2.2.2, 2.3.3.2 & 2.3.3.3). In terms of our studies, many students might not have had sufficient content knowledge when interacting with the diagrams representing biochemical processes to: visualise the spatial arrangement of the dynamic network of biochemical processes that constitute a “cell soup”, as opposed to a system of processes occurring in rigid structures (Chapter 5); correctly interpret or reason about the glycolytic (Chapter 6) or complement (Chapter 7) reactions; or, to understand the symbolism or terminology representing these 2 latter processes. Thus as a potential remediation strategy, to aid students who lack the relevant content or prior knowledge to interpret a diagram, contextual information (e.g. text) should be included with the diagram (Lowe, 1996). For example, explaining why certain features are depicted in certain diagrams and why different representations are used to depict the same process (Kindfield, 1993/ 1994). However, the students in our studies were taught both the chemistry and symbolism of the individual reactions of the glycolytic pathway and the complement system. As a result, a sound content knowledge of reaction mechanisms did not prevent several students in our studies (Chapters 6 & 7) from showing visualisation difficulties. Thus, we realised the need to also address the other 2 factors of Schönborn’s model as possible sources of the difficulties, namely, students’ diagrammatic reasoning ability and misleading modes of representing the content knowledge as diagrams (Schönborn *et al.*, 2003 & 2002).

In terms of students’ reasoning ability, our results suggest that many students did not use the information provided on the glycolytic diagram (Chapter 6, Fig. 6.1), e.g. the chemical structures, wording and stoichiometry, to aid them in interpreting and visualising the glycolytic reactions. In addition, students did not reason about the nature of the complement reactions using the symbolism in the diagram (Chapter 7, Fig. 7.1), even though they possessed the content knowledge about the key reaction components. Therefore, these results indicate that one source of students’ visualisation difficulties could be an interaction between the reasoning factor and the students’ conceptual knowledge, namely that they were not reasoning with the diagram and their conceptual knowledge of relevance to the diagram, when visualising the biochemical process (Schönborn *et al.*, 2003 & 2002). Thus, the implications of this are that students may have the required content knowledge, but a poor ability to reason with the symbolic information provided on each diagram (Schönborn *et al.*, 2003 & 2002).

Thus, although we took cognisance of the role of the students' conceptual knowledge, their reasoning ability, and how these 2 factors influenced their interpretation of the diagram and visualisation of the biochemical process, the visualisation difficulties we identified in our studies (as mentioned above) were primarily attributed to the diagram design factor (Schönborn *et al.*, 2003 & 2002). For example, artists sometimes use analogies between the functional nature of a process (e.g. cyclic) and the manner in which they depict the process in a diagram (e.g. in a circle). However, this becomes a problem when some students take this analogy or symbolic representation literally and think that these processes occur in these structures within the cell. Thus, this suggests that although the conventional symbolic manner of depicting certain biochemical processes in diagrams aids in helping students to link a particular process to its name (e.g. linear, cyclic and cascade) and function, by not giving students multiple representations and/ or explanations of these diagrams, leads some of them into thinking these processes occur in these spatial arrangements within cells (Chapter 5). In addition, data collected from students in the glycolysis (Chapter 6, using Fig 6.1) and complement (Chapter 7, using Fig. 7.1) studies also revealed that the diagrams were a major source of the visualisation difficulties, in terms of the symbolism used and visiospatial characteristics employed in designing these textbook diagrams. For example, the use of the "+" sign, arrows and the visiospatial layout of the symbolic glycolytic diagram elements (Chapter 6, Fig. 6.1), as well as the symbolic lettering used to represent complement molecules (Chapter 7, Fig. 7.1), were shown through student responses to mislead and confuse some students in these studies. Thus, the diagram as a possible source of these visualisation difficulties, in terms of visiospatial layout and symbolism employed, will now be considered in terms of suggestions for diagram design.

Since the diagram factor (Schönborn *et al.*, 2003 & 2002) is suggested to be a major source of the visualisation difficulties (Chapters 5- 7), then the students' ability to reason with these diagrams is likely to be hampered, no matter how tuned their reasoning skills might be. However, if students are taught or prompted to relate the information they extract from a diagram to their conceptual knowledge, by using their diagrammatic reasoning skills, any misinterpretations that may arise from interacting with a particular diagram may be overcome. In terms of the symbolism used in diagram design, key or contextual information explaining the use of the symbolism and the conventional manner in which processes are drawn, might be useful for aiding students to visualise the process and

reaction mechanisms. This suggestion is supported by Ametller and Pintó (2002), who have also shown that although students do not read captions until they are required to do so, these captions have aided students in establishing a context to interpret the image, and to assign correct meanings to the graphical elements. Thus, the suggestion by Phillips and Quinn (1993), of using more familiar symbols and standardising the conventions used in flow diagram design, might be a possible remediation technique.

Apart from this confusing or misleading use of symbolism in the diagrams used in our studies (Chapters 6 & 7), the visiospatial characteristics making up the structure of the diagram were also shown (as discussed earlier) to be a source of some student difficulties (Chapters 5- 7). The importance of visiospatial characteristics in diagram design was discussed in detail in the literature review (Section 2.2.1, Chapter 2). The visiospatial characteristics employed in diagram design are, for example, the placement of diagram elements, their connection with lines or arrows, and surrounding them with a common boundary (Winn & Solomon, 1993). Visiospatial characteristics have been shown to be important in diagram design (Winn & Solomon, 1993) and in allowing the diagram user to group or distinguish diagram elements from each other (Cheng *et al.*, 2001). Therefore, in order for students to successfully interpret diagrams, such as those representing biochemical processes, they need to correctly interpret the meaning behind the use of the visiospatial characteristics. For example, determining which elements of the diagram are related or directly influence each other, as in some cases the spatial arrangement of the diagram elements depicts their connection and the sequence of events that occur, and not the natural state of the system (Lowe, 2001, pers. comm.). However, in other cases, the visiospatial characteristics or diagram structure does not always indicate how or if diagram elements are related conceptually or functionally (Lowe, 1997).

If the visiospatial characteristics of the diagram do not clearly highlight the key relationships and foster effective diagram interpretation, the use of cues (Lowe, 1997) and contextual information to guide the diagram user in correctly interpreting the diagram (Lowe, 1996 and Kindfield 1993/ 1994), as mentioned earlier, may be used. Another possible remediation strategy is the use of multiple representations. These have been supported by a number of authors as being useful in promoting reasoning and transfer when the same information is presented in different diagrams (e.g. Stern *et al.*, 2003, Seufert, 2003), and, the use of one diagram has been shown to aid students in interpreting

another type of diagram of the same image (Ametller & Pintó, 2002). Evidence for the support of multiple representations as an intervention and remediation strategy was also shown in 2 of our studies. In this regard, the presence of an alternative diagram of the glycolytic pathway (Chapter 6, Fig. 6.8) was shown to aid some students in correcting their previous difficulties, and some students after multiple exposures (i.e. in written probes and then interviews, see Chapter 5, Fig. 5.2) to non-conventional representations of a process, were shown to recognise the more functional features of a process and not rely solely on literal interpretation to classify processes.

This general discussion has, thus far, focused mainly on students' visualisation difficulties (Chapters 5- 7), possible sources of the difficulties and potential remediation strategies. Apart from the identification of student difficulties from their responses to various written and interview probes, there were, however, cases in Chapter 5 where we noticed a difference in students' expression models or external representations of how they visualised the spatial arrangement of biochemical processes occurring *in vivo*. In this regard, some students diagrammatically favoured representing linear processes in a "straight line", cyclic processes in a "circle" and cascade processes in a "step-wise" fashion, but they correctly verbally conveyed that they did not visualise these processes occurring within these conventional spatial arrangements within the cell. In contrast, other students showed difficulties by both verbally and diagrammatically expressing that they thought these processes occurred in the conventional structures (or spatial arrangements), that they are represented in textbooks and other literature. Therefore, we identified 2 types of students' responses, whereby both sets of students drew biochemical processes in the conventional manner, but the one group verbally expressed that they did not think these processes occurred in these conventional textbook spatial arrangements within cells, while the other group verbally described that they did. An interesting observation that may explain why both groups of students in our sample diagrammatically represented processes in the conventional textbook representations, was found in a study by Brna *et al.* (2001). These authors have stated that students only generate new diagrammatic representations in a few cases, possibly because the educational system does not leave much room for this, therefore, students should be encouraged during instruction to generate their own diagrams to represent a situation (Brna *et al.*, 2001). We may apply this latter suggestion as a possible intervention and remediation strategy, to correct and circumvent students' literal interpretation of the conventional mode of biochemical process representation. As a result,

we may move closer to a students' mental model of a situation because, as Reiss and Tunnicliffe (2001) have stated and we have shown, there is a difference between a student's mental model and expressed model, and a researcher therefore needs to elicit more than one of their expressed models. We did this in all our student investigations by giving the students written probes (written representation) and asking students to draw a diagram (diagrammatic representation) and then probing them on it in an interview situation (verbal representation). However, in Chapter 5, we noticed not only differences between students' mental and expressed models, but also differences between the different types of expression models (e.g., verbal compared to diagrammatic representations).

These differences and similarities between students' expressed models is diagrammatically represented and incorporated into a model of visualisation in Fig. 8.1 below. This model has evolved from the interpretive framework of the research process employed in this study (Methods Chapter, Fig. 3.1), for investigating students' visualisation of biochemical processes. Through the analysis of our results in Chapters 5- 7, we subsequently propose that our data forms preliminary empirical evidence for this model of visualisation (Fig. 8.1).

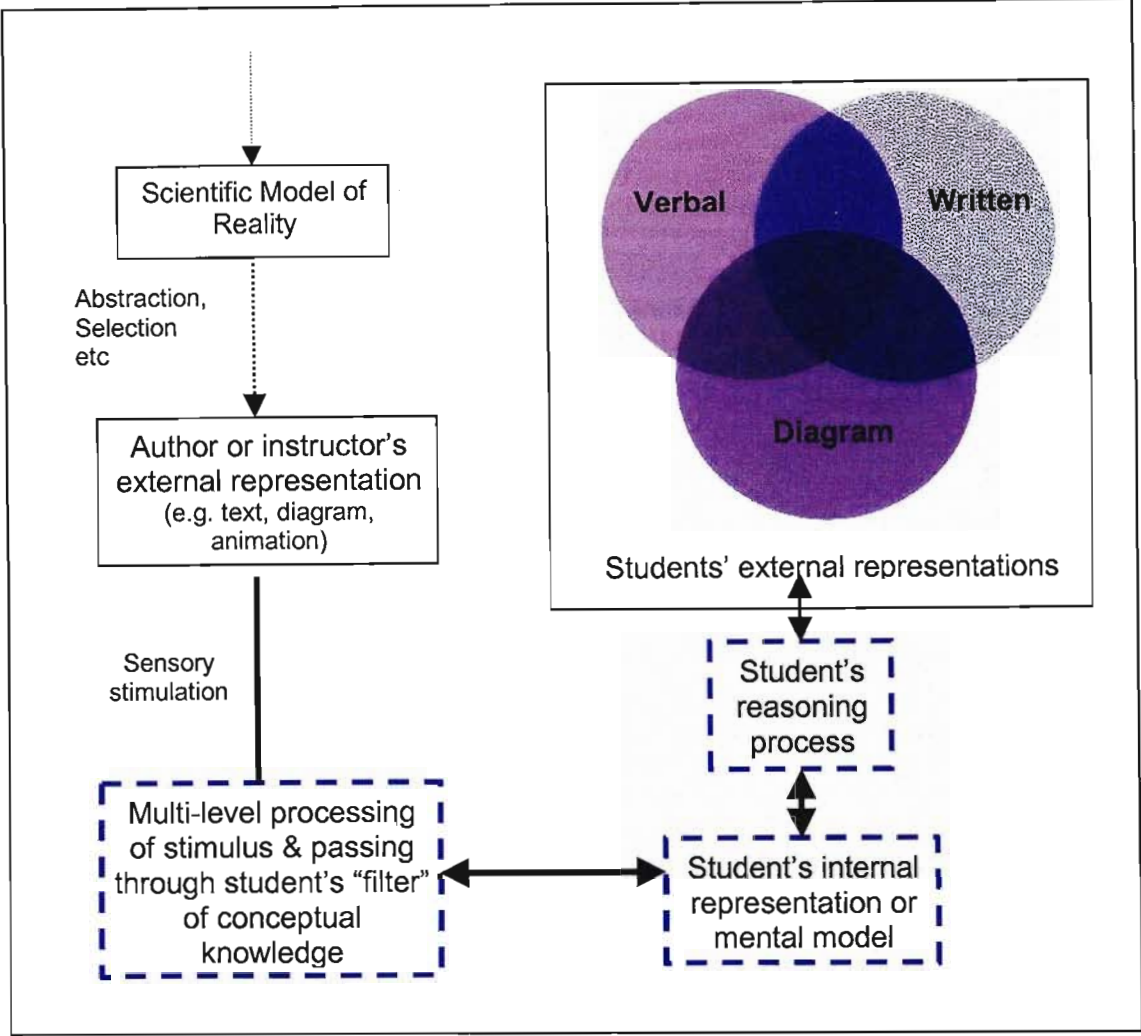


Figure 8.1: Proposed model of the process of visualisation

The visualisation model (Fig. 8.1), that we propose, is based on the view that the process of visualisation (discussed in the literature review, Section 2.3.2) incorporates the entire process from receiving a sensory stimulation, to the expression of an individual's mental model of the situation. This is in agreement with the view of McCormick *et al.* (1987) who suggest visualisation involves the perception, interpretation, use and communication of visual information. The external representation responsible for the sensory stimulation (e.g. diagram), which is perceived by an individual, reflects to a large extent the author's mental model of the depicted situation (Lowe, 2001, pers. comm.). We propose that an individual passes the extracted information from the external representation through their "filter" of existing conceptual knowledge (based on constructivist theory, Chapter 1 & Chapter 3, Section 3.2) and thereby constructs an internal representation, stored as either

an image or proposition (based on the imagery debate, Chapter 2, Section 2.3.3.1). The multi-level processing of the stimulus (Fig. 8.1) also incorporates the dual coding theory of Mayer (e.g. 2003). This is because the extracted information would be processed in either the verbal or visual channel, depending on the format of the sensory stimulation (e.g. text and diagram, respectively), and the individual would then form referential links between these 2 representation systems and between their prior knowledge in an iterative fashion (e.g. Mayer, 2003). In this regard, we represented all internal processes occurring in the diagram user's mind (see Fig. 8.1) in blue dashed boxes, and used double-headed arrows to represent the iterative and continual processes that are involved in the construction and modification of an individual's mental model of a situation.

Furthermore, our studies required students to express (elements of) their mental model of a depicted situation, thus allowing us to identify any difficulties the individual may have had (Chapters 5- 7). At the same time, this enabled us to compare the information content of their expressed models or external representations for discrepancies. The Venn diagram in Fig. 8.1, therefore, illustrates that by eliciting the 3 types of student's external representations, we may determine whether all 3, none, or 2 of their expressed models overlap or communicate the same information. This triangulation approach, also affords a measure of validity to our studies since student difficulties exposed through eliciting one type of external representation, may be an artefact of that representation system, and not a true reflection of their internal model or understanding of the situation. We, therefore, suggest that at least 2 types of external representation should be exposed in any study that investigates an individual's understanding of a situation, since not only might there be a difference between an individual's expressed and internal/ mental model (Reiss & Tunnicliffe, 2001), but we have also found differences between the different types of expression models or representations (Chapter 5).

In summary, the studies reported on in this dissertation have achieved the following major specific findings in terms of the research questions posed:

- 1.) From a questionnaire to biochemistry experts, we have identified an extensive list of generic and a limited number of distinguishing functional features that characterise linear, cyclic and cascade biochemical processes. Although this list requires further clarification, it promises to make an important contribution to

learners, teachers and researchers to discuss and understand the nature of biochemical processes in metabolic systems;

- 2.) Attempts to clarify current knowledge of the complement system revealed a deficiency in immunology experts' knowledge as to the functional relationship between the 2 complement pathways and, therefore, highlighted the need for further experimental laboratory work;
- 3.) Some students literally interpreted diagrammatic representations of the functional characteristics of biochemical processes (e.g. cyclic processes occur in circles), as the spatial arrangement of the intermediates within cells;
- 4.) In some cases, differences between individual student's expression models or external representations i.e. verbal, written and diagrammatic were identified. Whereas some students' diagrammatically represented biochemical processes in the conventional textbook spatial arrangements, they correctly verbally expressed that they did not visualise these processes literally occurring as such, within cells;
- 5.) Students also showed difficulty in visualising the chemistry of individual reactions in the glycolytic (SR and IR classes) and complement pathways (EC class) as represented by textbook diagrams;
- 6.) The possible sources of students' visualisation difficulties were primarily thought to be the misleading use of symbolism and visiospatial characteristics in the diagrams, as well as deficiencies in students' conceptual knowledge and reasoning ability. This constituted further empirical evidence for the model of Schönborn et al. (2003 & 2002);
- 7.) A major implication of the results in this dissertation is the need to improve diagram design through the use of clearer and more intuitive symbolism, the standardisation of conventions, and improvement of the visiospatial properties of diagrams;
- 8.) The overall results and research process used in the studies reported in this dissertation, lent itself to the preliminary development of a model of visualisation that requires further empirical testing, but promises to enhance future research into researchers' understanding of the highly complex and cognitive processes involved in individuals' visualising biochemical processes in living systems.

Our results, as summarised by points 1- 8 above, have stimulated us to raise several new questions that could be addressed by the following further research:

- 1.) The extension of the expert study (Chapter 4) to include data (already collected) on experts' functional features of a wider range of biochemical processes (e.g. branched, shuttle and electron transport processes), to further supplement our preliminary features for linear, cyclic and cascade biochemical processes;
- 2.) Investigation into whether incorporating the identified distinguishing and generic functional features of linear, cyclic and cascade biochemical processes into courses on metabolism will have a positive effect on students' and experts' understanding, visualisation and communication of these processes;
- 3.) The further evaluation of whether multiple representations of biochemical processes serve as effective remediation tools for students' visualisation difficulties with biochemical processes, particularly in terms of their spatial arrangement in cells and their chemistry (Chapter 6, Fig. 6.1 & 6.9, provided preliminary evidence);
- 4.) The formulation of a list of guidelines for diagram designers and textbook authors, based on the evidence of the studies reported in this dissertation (Chapters 4- 7), knowledge from the literature, and the data collected from future studies suggested in points 1- 3 above;
- 5.) The formulation of a list of guidelines for the use of diagrams for the teaching and learning of biochemical processes;
- 6.) The further development and testing of the proposed model of visualisation (Fig. 8.1), by including more studies in a wider context, utilising representations and instruction tools other than diagrams (e.g. animations and text), and investigating more deeply the different stages of the visualisation process;
- 7.) Further immunological laboratory research to investigate whether the alternative and classical complement pathways (Chapter 7) are, or are not, functionally linked via complement protein C3b.

In conclusion, our results from the student investigation reported in Chapters 5- 7, show that experts should not assume that diagrams they have designed for textbooks will necessarily be effective as teaching and learning tools for improving students' understanding and visualisation of biochemical processes in living systems. In this regard, the way experts' interpret diagrams is different from the way novices' read them, mainly

because of difference in prior conceptual knowledge. There is an urgent need to clarify the meaning of the different biochemical processes so that experts and novices can discuss metabolism more efficiently. As one expert remarked in response to our questionnaire on the features of biochemical processes: "Gee, makes you think doesn't it?". Thus, we believe that our work has laid a solid foundation for exciting and creative future research opportunities in the area of biochemical processes, and the use of diagrammatic representations of such processes for teaching, learning and research.

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Appendix A

Masters Project on Student Conceptual and Reasoning Difficulties with Biochemical Processes in Cells

Questionnaire

Dear Biochemist

I am currently studying for my M.Sc. degree in biochemistry education at the University of Natal (Pietermaritzburg), South Africa under the supervision of Dr Trevor Anderson. I am investigating students' conceptions of the different types of biochemical processes that constitute cellular metabolism. Although biochemistry textbooks illustrate examples of the different biochemical processes there are only limited attempts to describe and compare the various distinguishing functional features of each type of process.

I would greatly appreciate it if, based on your expertise and knowledge, you were prepared to complete the following questionnaire so that I can compare biochemistry students' understanding of these processes with that of experts in the field. Kindly also forward this email to other colleagues.

As for all our science education research, the identity of individuals responding to this questionnaire, as well as the name of their institutions, will be kept confidential.

Thank you in advance for using some of your valuable time to complete this questionnaire. We appreciate it very much.

Yours sincerely

Tracy Hull

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Instructions:

Please feel free to make your responses to the questions below as long and as detailed as you wish.

Please return the questionnaire by email, as an attached file, as soon as possible to:

Ms. Tracy Hull at: 962082161@students.unp.ac.za **OR**

Dr Trevor Anderson at: anderson@nu.ac.za

What in your view are the distinguishing functional features of:

A linear process, e.g. the conversion of glucose to fructose 1,6 bisphosphate in glycolysis?

A cyclic process, e.g. the citric acid cycle?

A parallel-coupled process, e.g. as in ATP coupling in the hexokinase reaction?

A cascade process e.g. the complement pathways of the immune system?

A branched process e.g. the conversion of pyruvate to either lactate or acetyl CoA?

A process with a “split”, e.g. the conversion of fructose 1,6 bisphosphate to glyceraldehyde 3-phosphate and dihydroxyacetone phosphate in the aldolase reaction in glycolysis?

A shuttle process, e.g. the malate-aspartate shuttle?

An electron transport process, e.g. in oxidative phosphorylation or in the reduction of ribonucleotides by ribonucleotide reductase?

Any further comments?

Appendix B

Use the diagram sheet provided below to answer the following questions

(a). Which diagram(s) represent(s) linear process(es)?

Circle your choice(s): 1 2 3 4 5 6 7 8 9 10 11

I made the above choice(s) because linear processes have the following characteristics:

(b). Which diagram(s) represent(s) cyclic process(es)?

Circle your choice(s): 1 2 3 4 5 6 7 8 9 10 11

I made the above choice(s) because cyclic processes have the following characteristics:

(c). Which diagram(s) represent(s) cascade process(es)?

Circle your choice(s): 1 2 3 4 5 6 7 8 9 10 11

I made the above choice(s) because cascade processes have the following characteristics:

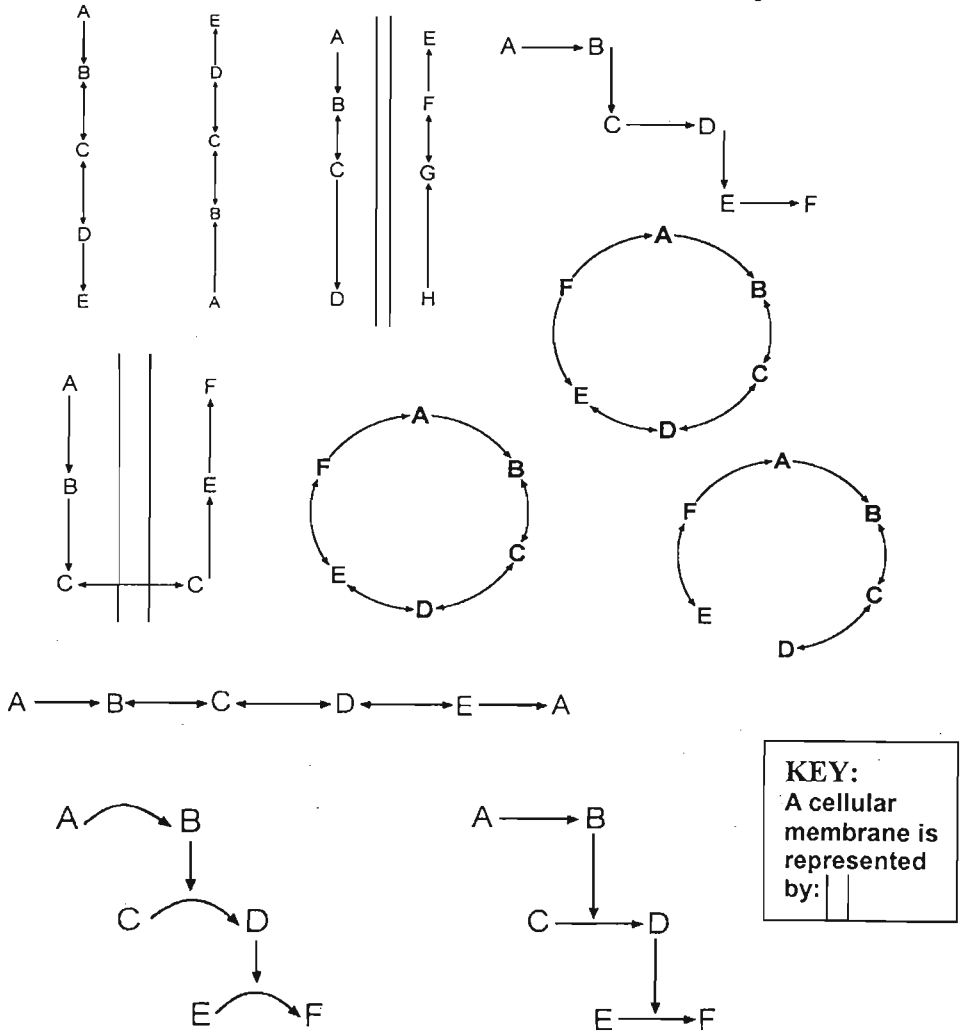


Figure 5.2 Multiple-choice type probes (excluding bullets under (a) – (c)) given to second year students to investigate any literal interpretations of representations of biochemical processes

Appendix C

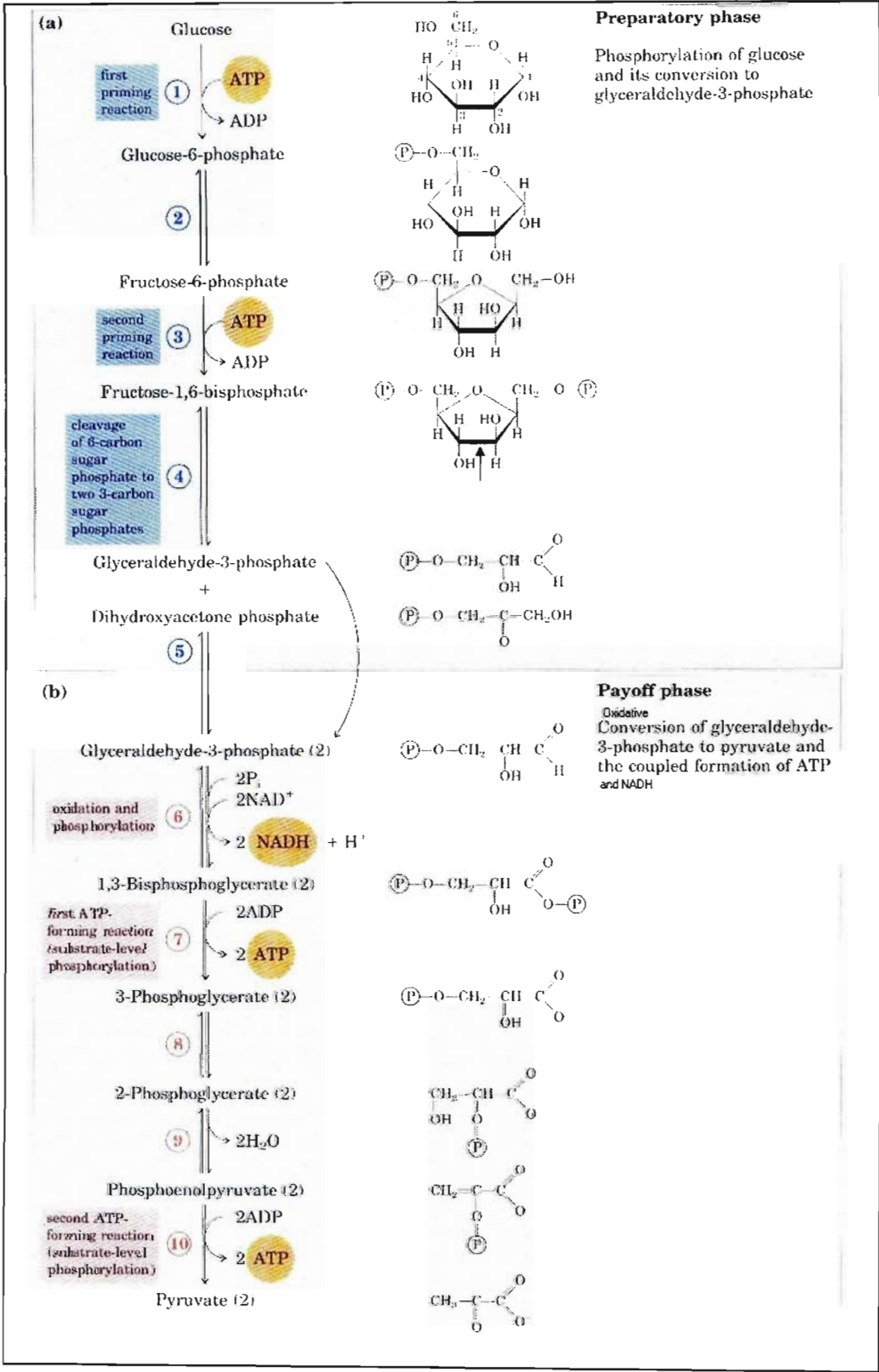


Figure 6.1 The two phases of glycolysis (Lehninger, 2000).

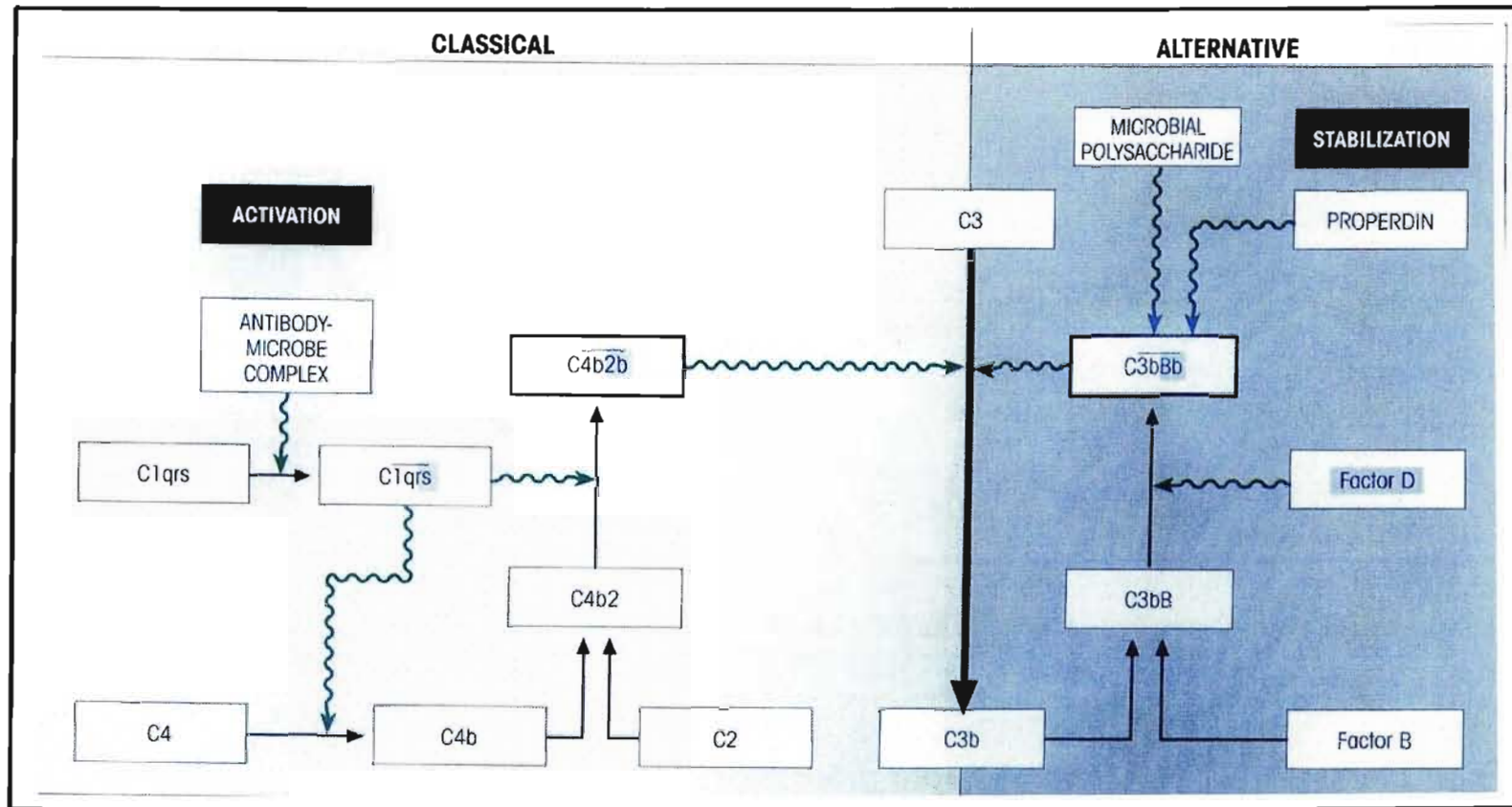


Fig. 7.1 Comparison of the classical and alternative complement pathways. (Roitt, 1997, Reprinted by permission of Blackwell Science, Inc.).